Review

The Pathology of Pathogenic Theileriosis in African Wild Artiodactyls

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Abstract

The published literature on schizont-"transforming," or pathogenic theileriosis, in African wild artiodactyls is dated and based on limited information. Here the authors review the taxonomy, diagnosis, epidemiology, hematology, pathology, and aspects of control in various species. Molecular studies based on 18S and 16S rRNA gene sequences have shown that African wild artiodactyls are commonly infected with diverse *Theileria spp.*, as well as nontheilerial hemoprotozoa and rickettsia-like bacteria, and coinfections with pathogenic and nonpathogenic *Theileria* species are often recorded. Although theileriosis is still confusingly referred to as cytauxzoonosis in many species, the validity of a separate *Cytauxzoon* genus in artiodactyls is debated. The epidemiology of theileriosis is complex; the likelihood of fatal disease depends on the interplay of parasite, vertebrate host, tick vector, and environmental factors. Roan calves (*Hippotragus equinus*) and stressed animals of all host species are more susceptible to fatal theileriosis. Even though regenerative anemia is common, peripheral blood piroplasm parasitemia does not correlate with disease severity. Other than anemia, common macroscopic lesions include icterus, hemorrhages (mucosal, serosal, and tissue), fluid effusions into body cavities, lung edema, and variably sized raised cream-colored foci of leukocyte infiltration in multiple organs. Histopathologic findings include vasocentric hyperproliferation and lysis of atypical leukocytes with associated intracellular schizonts, parenchymal necrosis, hemorrhage, thromboembolism, and edema. Immunophenotyping is required to establish the identity of the schizont-transformed leukocytes in wild ungulates. Throughout the review, we propose avenues for future research by comparing existing knowledge on selected aspects of theileriosis in domestic livestock with that in African wild artiodactyls.

Keywords

Studies on pathogenic theileriosis in African wild artiodactyls are heterogenous with inconsistent findings. The reported pathology is dated and based on limited information. Here we summarize and analyze the available literature to identify research gaps in the current state of knowledge.

Theileria parasites belong to the order Piroplasmida.[102,161] They are obligate intracellular hemoprotozoan parasites that are transmitted by ixodid ticks.[129] The Piroplasmida are classified into 3 families based on the vertebrate cells in which they develop[132]: Theileriidae infect mammals, first developing in leukocytes, then erythrocytes; Babesiidae infect mammals and some birds but develop only in erythrocytes; Hemohormidiidae infect fish and reptiles, where they develop in nucleated red blood cells.[132] The word *piroplasm* derives from the small pyriform stages that develop in vertebrate erythrocytes.[132]

Two piroplasmid genera, *Theileria* and *Babesia*, are responsible for some of the most economically important diseases of domestic and wild animals.[102,136,137] *Theileria* species are known to cause theileriosis in a range of domestic and wild ungulates.[102] While morbidity and mortality rates vary across

Theileria and host species,[102] theileriosis is the most significant tick-borne disease to affect domestic ruminants in Africa.[129] *Theileria parva* alone is suggested to be responsible for the death of >1 million cattle annually and a monetary loss of at least US$300 million per year.[111,126] Aside from this loss, the reductions in growth rate and productivity of infected ruminants and the costs of disease control and treatment all significantly limit the expansion of livestock farming and its contribution to the economy of the continent.[80,94,101,129]

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Historically, theilerial parasites were researched in African wild artiodactyls because they were considered to be likely reservoirs for *T. parva* infection in cattle.* This is indeed the case with African buffalo (*Syncerus caffer*), with buffalo-derived *T. parva* being the causative agent of Corridor disease, an acutely fatal disease of cattle in eastern, central, and southern Africa, especially south of the Zambezi River.[60,93,129,153,198]

The impact of theileriosis on the wildlife industry is less well documented. Although difficult to quantify in terms of its exact economic impact in Africa, wildlife tourism currently generates significant government revenue and foreign exchange, which contribute to employment creation on the continent, particularly in eastern and southern Africa.[40,133,136] As a result, a number of countries now target wildlife tourism as a primary growth sector with significant potential for socioeconomic transformation and poverty alleviation.[133] While African wild ungulates carry diverse hemoprotozoa, such as *Babesia*, *Theileria*, and *Trypanosoma*, as well as rickettsia-like bacteria, in particular *Anaplasma* and *Ehrlichia spp.*,† they are normally asymptomatic, unless stressed.[28,142,145] However, wild artiodactyls may be carriers of potentially pathogenic *Theileria spp.*[26,28,74,122,123,128,129,169] and may also experience pathogenic theileriosis with resultant decreases in population size; this is especially pertinent in rare and endangered species, such as roan (*Hippotragus equinus*) and sable (*Hippotragus niger*) antelope (Tables 1 and 2).[136,137,175]

Traditionally, and for the purpose of this review, *Theileria* species and strains are classified as pathogenic (schizont "transforming") or benign to mildly pathogenic ("nontransforming").[102,166] Pathogenic theilerias typically cause disease via schizont-induced alteration of the target mammalian host leukocyte transcriptome to induce blastogenesis, uncontrolled proliferation, widespread leukocyte dissemination, leukocytolysis, acute disease, and death of the vertebrate host.[102,185] Some pathogenic species in cattle have been particularly well researched. Examples include cattle-derived *T. parva*, which causes East Coast fever (ECF) in eastern, central, and southern Africa, and *T. annulata*, which causes bovine tropical theileriosis in North Africa, southern Europe, and large parts of Asia.[17,90,94,126] Pathogenic species/strains in African wild artiodactyls include *Theileria sp.* (sable),[128,175,176] *Theileria sp.* (gray duiker),[128] and *Theileria sp.* (greater kudu)[128] (Tables 1 and 2).

In contrast, certain *Theileria spp.* in cattle (eg, *T. velifera* and *T. sinensis*) are considered benign, since they have never been associated with disease.[87,102] Similarly, *T. ovis* and *T. separata* are examples of benign, nontransforming species in sheep and goats, although there is significant confusion about the taxonomy of nonpathogenic *Theileria* species in small ruminants.[89,102,188] These species have also been reported in

dogs and diverse wild artiodactyl species (Table 2),[28,102] although the validity of this conclusion is debatable (see Taxonomy section for details).

The classification of *Theileria* species as pathogenic or benign is an oversimplification because some parasites are not consistently pathogenic or benign within and/or among mammalian host species. For example, although generally regarded as benign parasites of cattle, *T. taurotragi*, *T. mutans*, and *T. buffeli/orientalis* are occasionally pathogenic but typically nontransforming.[87,102] In these instances, clinical disease and even rare mortalities occur due to piroplasm-induced acute hemolytic anemia, which is preceded by insignificant intraleukocytic schizogony.[88,91,102]

To complicate matters further, the normally benign *T. taurotragi* (in addition to pathogenic cattle-derived *T. parva* and *T. annulata*) is known to cause bovine cerebral theileriosis/turning sickness in young African shorthorn cattle.[14,33,92] This is an atypical acutely fatal form of pathogenic theilerial infection characterized by significant schizont-associated leukocyte transformation, proliferation, and invasion of the brain and spinal cord.[14,33,92,187] *Theileria taurotragi* also causes pathogenic theileriosis in eland (*Tragelaphus oryx*), characterized by schizont-induced pathology in diverse organs and tissues (Tables 1 and 2).[21,54,104]

The *Theileria* genus is characterized by a complex 2-host life cycle (Fig. 1).[129] The life cycle of pathogenic *Theileria* parasites in domestic ruminants has been comprehensively reviewed.[7,90,94,129,162] Specifically, *T. parva* and *T. annulata* life cycles have been deduced from the ultrastructural characterization of their developmental stages in vertebrate and tick host cells.[7,112,129,163] In contrast, there are very few ultrastructural studies on *Theileria* parasites in wild artiodactyls and their tick vectors.[43,75,200,201] As a result, little is known about the life cycle of *Theileria* species in these hosts. Studies have shown that asexual reproductive stages occur in the vertebrate host and tick, whereas the sexual phase occurs only in the tick midgut.[129,200] The biphasic life cycle in mammalian hosts includes an intraleukocytic developmental phase (pre-erythrocytic schizogony), which is followed by infection of erythrocytes to form piroplasms.[126,185]

Briefly, after ticks have fed on an infected vertebrate, some piroplasms escape from ingested erythrocytes in the tick midgut. They form female and male gametes, which fuse to produce nonmotile zygotes. The zygotes penetrate the intestinal epithelium, where they undergo meiosis and ultimately transform into motile kinetes. These are transmitted transstadially, ensuring persistence of infection during tick metamorphosis from larvae to nymphs to adults. Kinetes penetrate the gut wall, moving in the hemolymph to reach the salivary glands. Here they produce multinucleated sporoblasts in the cytoplasm of acinar epithelial cells. During feeding, sporogony is completed, and hundreds of infective progeny sporozoites are inoculated with tick saliva into the mammalian host. Sporozoites enter mammalian leukocytes and divide asexually (schizogony) to form multinucleated schizonts. These induce uncontrolled pseudo-neoplastic proliferation of leukocytes, which

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*References 9, 11, 21, 32, 54–57, 59, 74, 98, 123, 124, 129, 159, 169, 171
†References 15, 16, 20, 26, 36, 52, 66, 69, 78, 83, 100, 109, 114, 118, 119, 130, 138, 143, 181, 191

Table 1. Pathogenic *Theileria* Species and Strains Reported in African Wild Artiodactyls.[a]

Artiodactyl Species: *Theileria* Species[b]	Age[c] and Sample Size	Tick Vector[d]	Geographic Region
African buffalo (*Syncerus caffer*)			
Theileria sp./spp. (BS, HP)	<2 y (n = 3)[e,10]	Not specified	South Africa
T. lawrencei (Kenya), now buffalo-derived *T. parva* (BS, TS of lymph nodes)	(n = 1)[f,24,25]	*R. appendiculatus*	Kenya
T. parva (buffalo-derived BV-1 stabilate) (BS, TS)	1–2 y (n = 1)[f,g,86]	Not specified	Zimbabwe
Gonderia lawrencei, now buffalo-derived *T. parva* (PM, TS)	2 mo (n = 2)[123]	Not specified	South Africa
Coke's hartebeest (*Alcelaphus buselaphus cokii*): *Theileria sp.* (TS of the spleen)	(n = 1)[h,26]	Not specified	Kenya
Eland (*Tragelaphus oryx*)			
Theileria sp. (BS and TS of the spleen)	(n = 1)[e,11]	Not specified	Kenya
Cytauxzoon taurotragi (BS, TS, HP)	1 yr (n = 1)[21,104]	*R. appendiculatus, R. pulchellus*	Kenya
Theileria sp. (eland) (BS, TS, HP, and CC)	4 mo (n = 1)[f,57,58]	*R. appendiculatus, R. pulchellus*	Kenya
Theileria taurotragi (BS, TS, HP)	4 mo,[f] 7 mo, 11 mo,[f] 5 y, 6 y (n = 5)[54]	*R. appendiculatus*	Kenya
Theileria taurotragi (HP)	(n = 1)[f,61]	*R. appendiculatus*	Kenya
Theileria sp. (TS)	(n = 1)[99]	Not specified	Tanzania
Theileria and/or *Cytauxzoon spp.* (BS, TS, CC)	6 mo[g] (n = 1)[170,199]	*R. appendiculatus*	Kenya
Giraffe (*Giraffa camelopardalis*)			
Cytauxzoon sp. (BS, HP)	(n = 1)[108]	Not specified	Imported from Namibia to South Africa
Theileria sp. (giraffe) (BS, PCR-RLB, and sequencing)	2 y (n = 1)[h,136]	Not specified	Imported from Namibia to South Africa
Giraffe (reticulated; *G. c. reticulata*): *Cytauxzoon* and/or *Theileria sp.* (BS; TS from lymph node, lung, and spleen; HP of the kidney)	2 y (n = 1)[e,h,26,27]	Not specified	Kenya
Greater kudu (*Tragelaphus strepsiceros*)			
Cytauxzoon strepsicerosi (BS, TS)	5 mo (n = 1)[123]	Not specified	Translocated within South Africa
Theileria sp. (greater kudu) (PCR-RLB and sequencing)	(n = 1)[128]	Not specified	Translocated within South Africa
Gray/common duiker (*Sylvicapra grimmia*)			
Cytauxzoon sylvicaprae (BS, TS, HP)	(n = 1),[123] 2 y (n = 1)[125]	Not specified, not specified	South Africa, South Africa
Theileria sp. (gray duiker) (BS, HP, PCR-RLB, and sequencing)	Adult (n = 1)[128]	Not specified	South Africa
Roan (*Hippotragus equinus*)			
Theileria sp. (sable) (BS, HP, PCR-RLB, and sequencing)	(n = 2)[128]	Not specified	Imported from West Africa to South Africa
Theileria sp. (sable) (BS, TS, HP, PCR-RLB)	1 mo (n = 2)[f,173–175]	*R. evertsi evertsi, R. appendiculatus?*	South Africa
Cytauxzoon sp. (BS, TS, HP)	13 d, 7 wk (n = 2)[193,194]	*R. evertsi evertsi? R. appendiculatus? B. decoloratus?*	South Africa
Sable (*Hippotragus niger*)			
Theileria sp. (sable) (BS, TS, CC, PCR-RLB, and sequencing)	Calf (n = 1)[3,176]	Not specified	South Africa
Theileria sp. (sable) (HP, PCR-RLB, and sequencing)	(n = 1)[128]	Not specified	South Africa
Theileria/Cytauxzoon sp. (BS)	Calves (n = ?)[181]	Not specified	South Africa
Theileria/Cytauxzoon sp. (BS)	7 d (n = 1)[h,193]	Not specified	South Africa
Theileria/Cytauxzoon sp. (BS)	133 d (n = 1)[194]	*R. evertsi evertsi? R. appendiculatus? B. decoloratus?*	South Africa

Table 1. (continued)

Artiodactyl Species: _Theileria_ Species[b]	Age[c] and Sample Size	Tick Vector[d]	Geographic Region
Tsessebe (_Damaliscus lunatus_)			
Cytauxzoon sp. (BS, HP, TEM)	3 wk (_n_ = 1)[75]	Not specified	South Africa
Theileria/Cytauxzoon spp. (method of establishing infection not specified)	(_n_ = ?)[181]	Not specified	South Africa
Theileria/Cytauxzoon spp. (method of establishing infection not specified)	Calves (_n_ = ?)[h,194]	_R. appendiculatus?_	South Africa

Abbreviations: BS, blood smear; CC, cell culture; HP, histopathology; PCR-RLB, polymerase chain reaction–reverse line blot hybridization assay; PM, postmortem; TEM, transmission electron microscopy; TS, tissue smear.

[a]The method of parasite identification, host age, sample size, tick vector, and geographic region are listed.

[b]Based on PCR-RLB, BS, TS, serology, PM, HP, TEM, and/or behavior in CC.

[c]Where the age is known.

[d]Where the tick vector is uncorroborated, the name of the vector is followed by a question mark.

[e]Animals were culled for the purpose of data collection.

[f]Experimentally infected animals (in all other cases, natural infection is implied).

[g]Nonfatal infection (in all other cases, fatal infection is implied).

[h]Pathogenic theileriosis/cytauxzoonosis was not confirmed as the cause of death.

disseminate to a variety of organs. The ability to induce hyperproliferation and phenotypic atypia in vertebrate host leukocytes sets pathogenic _Theileria spp._ apart from benign and most mildly pathogenic _Theileria spp._, as well as other Piroplasmida and apicomplexans.[65,185] Some schizonts undergo asexual reproduction (merogony) to form merozoites. These mature, induce leukocytolysis, and subsequently infect mammalian host erythrocytes to form piroplasms.

Taxonomy

Most of the available information in African wild artiodactyls is dated, and the disease is still confusingly referred to as cytauxzoonosis in many cases (Table 1).[128] Prior to modern molecular assays and methods to identify leukocyte phenotypes, investigators assumed that although _Theileria_ and _Cytauxzoon_ intraerythrocytic piroplasms were indistinguishable, intra- and extracellular theilerial schizonts differed in number and morphology from those of _Cytauxzoon_ species.[7,8,21,26,74,75,108,123,125] It was also assumed that theilerial pre-erythrocytic schizogony was restricted to lymphoid cells (as in ECF) as opposed to lymphoid and other cell types, including histiocytes (or histiocytoid leukocytes) and hepatocytes, as in cytauxzoonosis.[7,21,108,123,125,128] However, since the schizonts of most pathogenic theilerial species induce pseudo-neoplastic transformation of infected and uninfected cells, the prior designation of cellular phenotypes based on morphology alone was simply guesswork. Subsequently, leukocyte phenotyping has shown that certain transforming theilerial species, such as _T. annulata_ and closely related _T. lestoquardi_ in domestic ruminants, are capable of infecting and transforming a variety of leukocytes, including histiocytes.[19,71] As a result, the validity of a separate _Cytauxzoon_ genus in African wild artiodactyl species is uncertain, and some researchers consider _Cytauxzoon_ to be a synonym of _Theileria_ in these species.[23,97,128,181] For the purpose of this review, we refer only to _Theileria_ and theileriosis in African wild artiodactyls.

Phylogeny of _Theileria_ species has largely been elucidated with 18S ribosomal RNA gene sequence analysis, and ever-increasing numbers of _Theileria_ 18S rRNA sequences are available in the public DNA sequence databases. Currently, identification of _Theileria_ parasites in wildlife relies largely on the amplification of full-length or partial 18S rRNA genes and DNA sequence analysis.[47,63,103,128,136,167,184,192] Sequence analyses of nuclear[103] S5 and mitochondrial cytochrome c oxidase 1 genes[146] have been used to distinguish _Theileria spp._ from closely related to _T. parva_ but have not been widely applied to other members of the genus.

In a phylogenetic tree generated with approximately 1400 bp of the _Theileria_ 18S rRNA gene, _Theileria_ sequences from African wild artiodactyls are found in several clades (Fig. 2). _Theileria sp._ (sable) is a known pathogen of roan and sable antelope[128,175,176] that groups with theilerias from sheep, African antelope, and dogs. The _Theileria sp._ (sable) 18S rRNA sequence is most closely related to _Theileria_ species identified in tsessebe (_Damaliscus lunatus_)[28] and dogs[107] from South Africa. _Theileria separata_[160] and a _Theileria_ species identified in gray/common duiker (_Sylvicapra grimmia_)[128] also group closely with _Theileria sp._ (sable). Full-length 18S rRNA gene sequences are not available for all theilerias that have been identified to date, and more partial _Theileria_ 18S rRNA sequences are available in the public sequence databases. When phylogenetic trees are generated from smaller 18S rRNA sequence fragments, similar groupings are found, although the branching of the clades tends to be slightly different and probably less accurate, as fewer characters are considered.[102] Shorter _Theileria_ 18S rRNA sequence fragments that also

Table 2. African Wild Artiodactyl Species With Fatal Pathogenic Theileriosis at the Faculty of Veterinary Science, University of Pretoria, South Africa (2007–2018).[a]

Artiodactyl Species: Date[b]	Host Age	Hemoprotozoa and/or Bacteria *spp*. Identified via PCR-RLB
African buffalo (*Syncerus caffer*)		
3.2007	10 mo	*T. parva*
3.2007	10 mo	*T. parva*
Bushbuck (*Tragelaphus scriptus*): 5.2018	Subadult	*Ehrlichia/Anaplasma* catch-all, *Theileria/Babesia* catch-all, *Theileria* catch-all, *Babesia* catch-all, *Theileria sp.* (greater kudu), *T. buffeli*
Eland (*Tragelaphus oryx*)		
7.2014	Adult	*T. taurotragi, T. bicornis, T. buffeli*
4.2016	3–4 mo	*T. taurotragi, Theileria sp.* (sable), *Theileria sp.* (greater kudu), *T. bicornis, T. buffeli, E. ruminantium, B. bovis*
2.2017	Calf	*T. taurotragi*
Golden wildebeest variant of the blue wildebeest (*Connochaetes taurinus*)		
2.2013	—	*Theileria sp.* (sable), *E. ruminantium*
1.2014	3 wk	*Theileria sp.* (sable), *T. separata, T. buffeli*
Greater kudu (*Tragelaphus strepsiceros*): 4.2010	Calf	*Theileria sp.* (greater kudu), *Theileria sp.* (sable), *T. bicornis, T. buffeli*
Impala (*Aepyceros melampus*)		
7.2013	Adult	*T. bicornis, Ehrlichia/Anaplasma* catch-all
9.2013	Adult	*T. bicornis, Ehrlichia/Anaplasma* catch-all
7.2014	Adult	*T. bicornis, T. buffeli, Ehrlichia/Anaplasma* catch-all
Lechwe (*Kobus leche*)		
3.2016	Adult	*Theileria sp.* (sable), *T. separata*
4.2018	Adult	*Theileria sp.* (sable), *T. separata, Ehrlichia/Anaplasma* catch-all
Roan (*Hippotragus equinus*)		
6.2005	2 mo	*Theileria sp.* (sable)
6.2007	1 y	*Theileria sp.* (sable), *T. separata*
5.2018	2–4 wk	*Theileria sp.* (sable)
5.2018	2–4 wk	*Theileria sp.* (sable)
Sable (*Hippotragus niger*)		
10.2014	—	*Theileria sp.* (sable)
12.2015	—	*Theileria sp.* (sable), *T. separata, T. ovis, B. sable*
6.2016	6 wk	*Theileria sp.* (sable), *T. separata*
6.2016	4 mo	*Theileria sp.* (sable), *T. separata*
6.2017	Adult	*Theileria sp.* (sable), *T. separata, T. bicornis, Anaplasma sp.* (Omatjenne)
8.2017	4 d	*Theileria sp.* (sable)
9.2017	3 wk	*Theileria sp.* (sable), *T. separata*
Sable (Zambian; *H. n. kirkii*)		
3.2015	5–6 wk	*Theileria sp.* (sable)
Waterbuck (*Kobus ellipsiprymnus*)		
2.2016	Adult	*Theileria sp.* (sable), *T. separata, A. bovis*
	1 mo	*Theileria sp.* (sable), *T. separata*

Abbreviations: PCR-RLB, polymerase chain reaction-reverse line blot hybridization assay.
[a]For which PCR-RLB results are available. The host species, postmortem date, host age, and hemoprotozoa and/or bacteria species identified are listed.
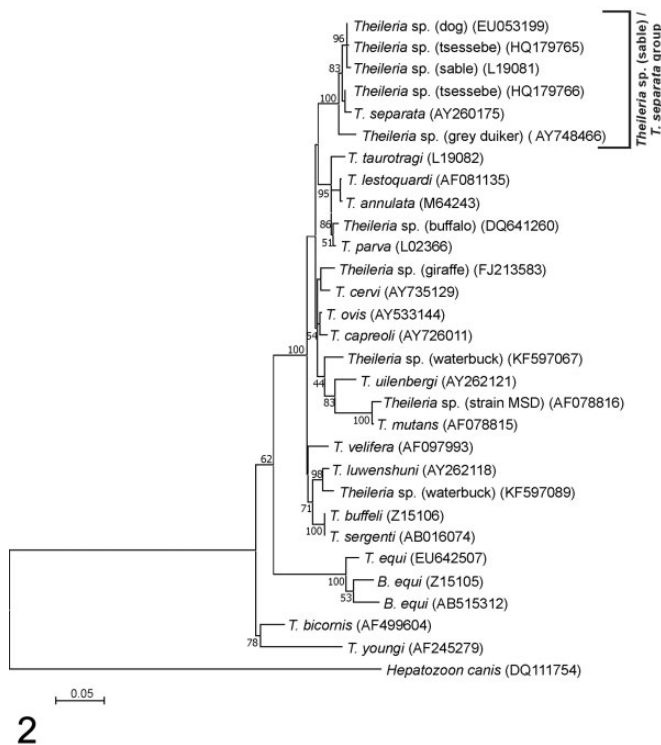[b]Dates are given as month.year.

group in the *Theileria sp.* (sable) clade have been identified in red hartebeest (*Alcelaphus buselaphus caama*),[167] blue wildebeest (*Connochaetes taurinus*),[192] buffalo,[103] and *Rhipicephalus evertsi evertsi* ticks collected from gemsbok (*Oryx gazella*) (Fig. 3).[184]

Theileria sp. (sable) has been shown to transform leukocytes[174–176,204]; however, *T. separata* is not known to be transforming, and nothing is known about the transforming capabilities of the other members of this group. Nevertheless, the *Theileria sp.* (sable) clade is closely related to a clade containing a number of *Theileria* species that are known to be pathogenic in domestic animals (Fig. 2) and are capable of transforming leukocytes. These include *T. parva* and *T. annulata*, as well as *T. lestoquardi* and *T. taurotragi*. Another member of this clade, *Theileria sp.* (buffalo), which infects buffalo,[2,164] is not known to cause disease in domestic ruminants but has been shown to transform lymphocytes.[205] Other well-recognized nontransforming *Theileria* species that infect cattle and buffalo, *T. mutans, T. velifera*, and *T. buffeli*, are more distantly related according to 18S rRNA gene sequence analysis.
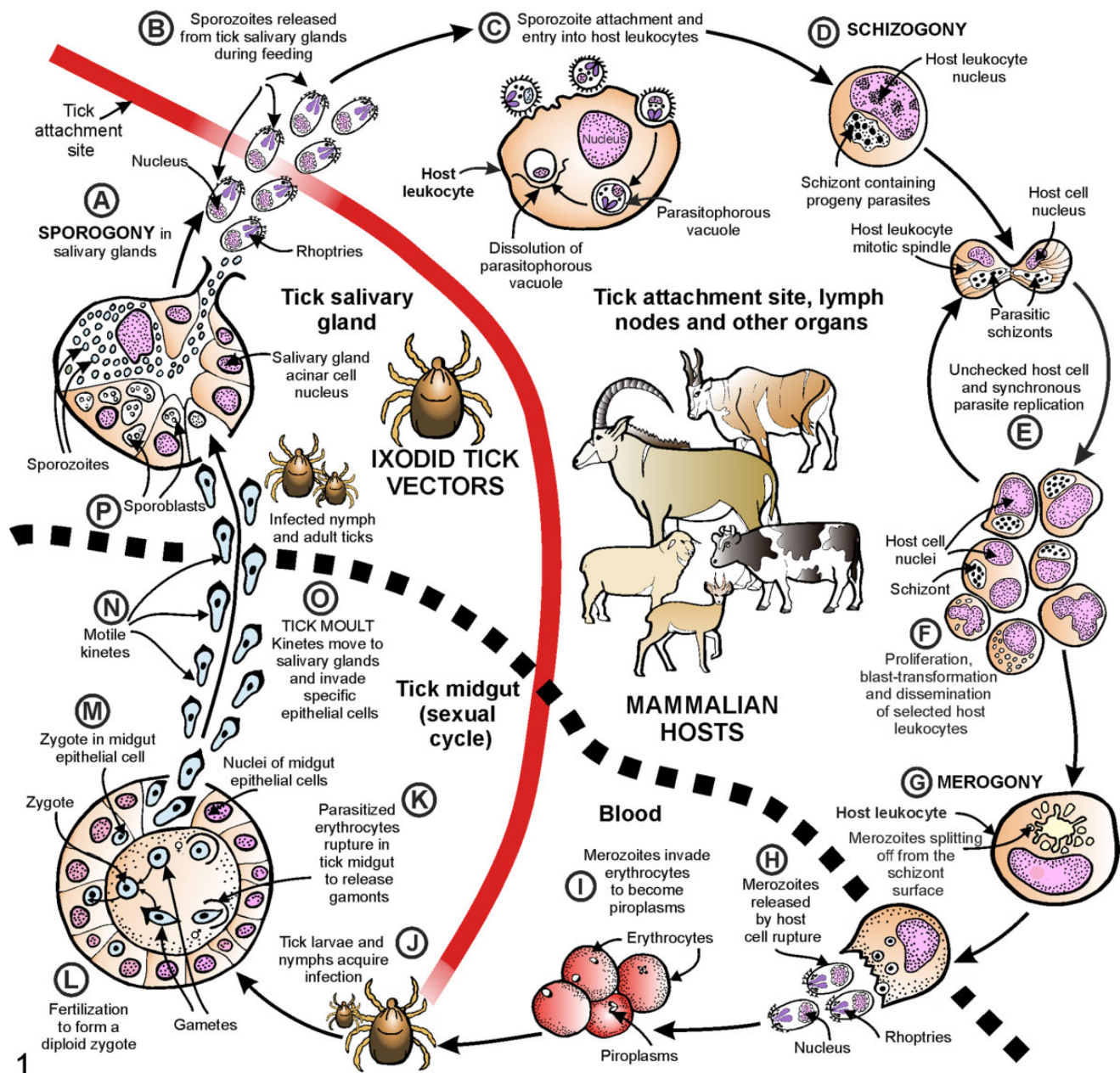
Figure 1. The life cycle of schizont-"transforming" *Theileria* parasites in domestic ruminants and their tick vectors. (A) During a blood meal, parasite sporogony is completed in specialized salivary gland acinar epithelium of a postmoult nymph or adult tick. (B) After feeding, mature sporozoites are injected into the new vertebrate host. (C) Sporozoites attach to and enter selected host leukocytes within the localized inflammatory reaction of the tick bite. (D) The parasites feed and develop into intracytoplasmic trophozoites, which then undergo schizogony to form multinucleated schizonts, or Koch blue bodies. (E) Schizonts associate closely with the host cell mitotic spindle apparatus, causing uncontrolled host cell and synchronous schizont proliferation with continuous distribution of schizont material to progeny leukocytes. (F) Schizonts induce selected host leukocytes to acquire a pseudo-neoplastic phenotype characterized by reversible blast transformation, self-perpetuating clonal expansion, and dissemination to a variety of lymphoid and nonlymphoid organs and the peripheral blood. These "transformed" leukocytes cause overwhelming systemic inflammation and acute fatal theileriosis. (G) A proportion of intracytoplasmic schizonts undergo merogony with the formation of progeny merozoites. Merozoites radiate in a rosette-like fashion from the surface of schizonts into the host cell cytoplasm, where they mature. (H) Leukocytolysis is induced upon merozoite exit. At this time, leukoproliferation decreases significantly. (I) Merozoites enter host erythrocytes to form pleomorphic intraerythrocytic piroplasms. (J) Tick larvae or nymphs feed on infected vertebrate hosts. (K) Erytholysis in the tick midgut causes piroplasms to be released, which develop into male and female gamonts. (L) Gametogenesis occurs in the midgut lumen to form male and female gametes, which fuse by syngamy to form a transitory spherical diploid zygote. (M) Zygotes penetrate the intestinal epithelium, where they increase in size over time. Meiotic division occurs in the zygote. (N) In synchrony with the process of tick moulting, zygotes develop into motile kinetes. (O) The kinetes move via the hemolymph to reach the tick salivary glands. (P) In the cytoplasm of specialized acinar epithelial cells, they undergo sporogony to form multinucleated sporoblasts. (A) When the tick engorges on a new host, sporogony is completed and the cycle is repeated. Figure adapted from Bishop et al,[17] Nene et al,[126] and stock image C022/0963.[42]

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Figure 3. Molecular phylogenetic analysis shows relationships among members of the *Theileria sp.* (sable) / *Theileria separata* group based on partial 18S RNA nucleotide sequences. A BLAST search was used to identify 18S sequences in the public sequence databases that are closely related to *Theileria sp.* (sable); sequence accession numbers are shown in parentheses. The same approach was followed as in Figure 2. Again, the tree with the highest log likelihood (−589.43) is shown. The parameter for the discrete gamma distribution was 0.8124, and 65.88% of sites were invariable. All positions containing gaps and missing data were eliminated from the 15-nucleotide sequences, giving 260 nucleotides in the final data set.
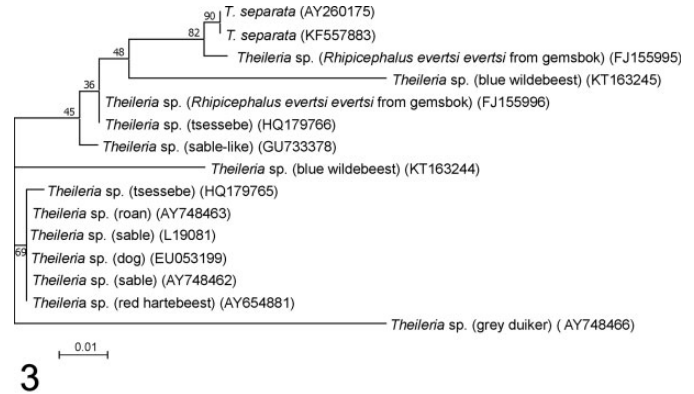
Figure 2. Molecular phylogenetic analysis of *Theileria* species based on near full-length 18S RNA nucleotide sequence data. The 30 sequences' accession numbers are shown in parentheses. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model.[177] The tree with the highest log likelihood (−5394.93) is shown. The percentage of trees in which the associated taxa clustered is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated via the maximum composite likelihood approach and then by selecting the topology with the superior log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.3259). The rate variation model allowed for some sites to be evolutionarily invariable (+I, 39.51% of sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were 1415 positions in the final data set. Evolutionary analyses were conducted in MEGA7.[84]

Other *Theileria* 18S sequences that are distinct from *Theileria sp.* (sable) have been identified in other African wild artiodactyls, including waterbuck (*Kobus ellipsiprymnus*),[47] greater kudu (*Tragelaphus strepsiceros*),[128] and giraffe (*Giraffa camelopardalis*).[136] These are more closely related to non-transforming *Theileria* spp. such as *T. mutans*, *T. velifera*, *T. buffeli*, and *T. ovis* from sheep and *T. capreoli* and *T. cervi* from deer in the Northern Hemisphere (Fig. 2).

Diagnosis

Differentiation of theileriosis from diseases that cause similar clinical signs, such as pyrexia, anemia, icterus, and enlarged peripheral lymph nodes, requires integration of clinical, epidemiologic, gross pathologic, light microscopic, and molecular data. The most important differential diagnoses for anemia and icterus in African wild artiodactyl species include babesiosis[‡] and anaplasmosis,[4,85,100,120,203] while heavy tick infestations have been associated with blood-loss anemia[113] and nonparalytic forms of tick toxicosis have been associated with pyrexia, anemia, and swollen lymph nodes due to lymphocytotoxicity.[44,121,156,174,180] Animals with nonpathogenic or subclinical infections may have occasional piroplasms in a blood smear and positive polymerase chain reaction (PCR) results.[136,137,142]

A reverse line blot (RLB) hybridization assay, originally designed for the simultaneous detection of pathogenic and nonpathogenic *Theileria* and *Babesia* species of cattle,[62] was adapted to include probes for the molecular confirmation of *Theileria* infections, including *Theileria sp.* (sable), in wildlife.[128] This RLB hybridization assay is based on 2 PCR assays: a *Theileria/Babesia* primer set and an *Anaplasma/Ehrlichia* primer set.[28,30,127,128,136,137] The PCR is followed by hybridization of the amplicons to species-specific probes to identify the species.[28,30,127,128] Two conservative probes for each of the *Babesia/Theileria* and *Anaplasma/Ehrlichia* groups are added to hybridize any species not identified by the species-specific probe set, so-called catch-all probes (Table 2).[103] A drawback of the RLB is that it will fail to detect novel species if the novel species occurs with a species in the probe set.[103]

African ungulates are commonly coinfected with not only a number of theilerial species, both pathogenic and benign,[102]

[‡]References 32, 106, 109, 136, 137, 181, 193, 194

but sometimes also with a variety of nontheilerial hemoparasites (Table 2).[34,102,103,147,167] The significance of these coinfections remains to be investigated,[127] including their potential influence on the sensitivity and specificity of the RLB hybridization assay.[102]

To date, *Theileria sp.* (sable) has been identified via RLB hybridization assays in random blood samples taken from a variety of healthy domestic and wild animals from various regions in southern Africa and Tanzania.[28,128,175,202] However, parasite 18S rRNA amplicons from cattle and buffalo samples that bind to the *T. velifera* probe also frequently bind to the *Theileria sp.* (sable) probe, suggesting that the *Theileria sp.* (sable) probe may cross-react with members of the *T. velifera* group.[103] Furthermore, 18S rRNA sequence analysis of samples from dogs[107] and tssessebe[28] that hybridized to the *Theileria sp.* (sable) probe revealed that although they contain a sequence with a 100% match to the *Theileria sp.* (sable) probe, there are other nucleotide differences that distinguish them from *Theileria sp.* (sable). A Primer-BLAST[195] analysis revealed that the *Theileria sp.* (sable) probe also has a 100% match in a *Theileria* 18S rRNA sequence obtained from a red hartebeest from Namibia[167] and single-nucleotide mismatches with sequences of *T. capreoli*, *T. ovis*, and *Theileria* identified in a variety of other hosts, including spotted deer (*Axis axis*), sika deer (*Cervus nippon*), and fox (*Vulpes vulpes*). Thus, the identification of *Theileria sp.* (sable) in cases of acute pathogenic theileriosis in African wild artiodactyls other than roan or sable (Table 2) may be due to cross-reactions of the probe with other previously unidentified but closely related *Theileria* parasites. In such cases, further sequence analysis is recommended to identify the parasite. It is clear that there is a need to develop a more specific *Theileria sp.* (sable) RLB probe, but until sequence information from closely related parasites from a large variety of wildlife hosts is available, it could be difficult to design a probe that does not cross-react with hitherto unrecognized species.

Other methods used for detection of the pathogenic *Theileria* species, including conventional PCR assays and real-time assays (reviewed in Mans et al[102]), are designed to be specific for the target parasites, and while they can be used to identify the target parasite in African wild artiodactyls, they cannot be used to detect novel parasites. An oligonucleotide multiplex suspension microarray has been developed for simultaneous detection of some of the *Theileria* and *Babesia* species included in the RLB hybridization assay.[157,158] This xMAP Luminex system shows great promise with the ability to multiplex up to 100 targets in a single reaction, but it will suffer from the same limitations as the RLB when it is applied to the detection of novel parasites in wildlife.

Epidemiology

The epidemiology of pathogenic theileriosis in African artiodactyls is complex and closely linked to the parasite's indirect 2-host life cycle. Disease severity is determined by the interplay of diverse theilerial parasite, tick vector, and mammalian
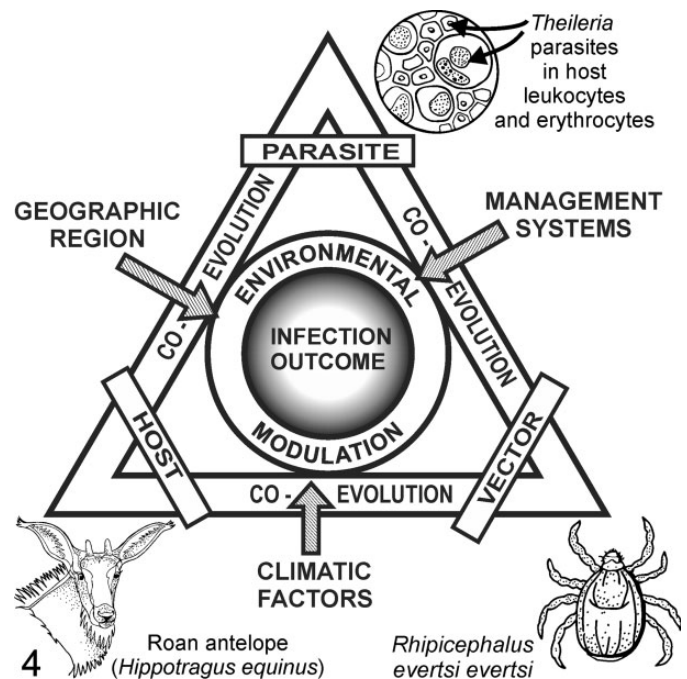
Figure 4. The complex interplay between parasite, vertebrate host, and tick vector factors and diverse environmental factors determines host susceptibility to clinical theileriosis. Parasite factors include variation in virulence among *Theileria* species and strains. Vertebrate host factors include age and immune status; tick vector factors include the effect of transstadial (vs transovarial) transmission of *Theileria* parasites on transmission efficiency. Environmental factors include climate (temperature and rainfall), geographic region, and management systems (eg, intensification of wildlife farming).

host factors (Fig. 4).[129] To complicate matters further, the environment, which includes numerous geographic, management, and climatic factors, also modulates the outcome of infection. This interplay is dynamic and can result in stable or unstable states.[64,76,94]

Endemic stability is a state of host-tick-pathogen interaction where infected ticks with low infection intensity constantly challenge calves and adults in a population, resulting in high levels of protective immunity in the majority of the population and hence low incidence of clinical disease.[64,94] Counterintuitively, anything that reduces the force of infection in such endemically stable areas, including seasonal fluctuations in vector abundance or erratic acaricide application, can lead to a state of endemic instability.[94] This is characterized by sporadic outbreaks of severe clinical disease and fatalities particularly in naïve hosts, such as exotic and crossbreeds of livestock and/or young domestic and wild artiodactyls.[94]

There has been a substantial increase in commercial wildlife ranching over the past 2 decades in South Africa.[175,189] The resultant confinement of wild artiodactyls in intensive breeding enclosures has resulted in increased tick burdens and tick-borne diseases, particularly pathogenic theileriosis in roan and sable calves.[128,173,174,189] Roan and sable antelope are rare and valuable, and despite successful breeding under intensive and

semi-intensive conditions, calf mortalities due to pathogenic *Theileria sp.* (sable) infection have negatively affected attempts to establish breeding herds and reintroduce animals into the wild.[174]

Parasite Factors

Disease severity is parasite and host associated and depends on a highly specific parasite–host cell relationship that differs among individuals, breeds, and species of hosts and parasites.[1] Although the infective dose of pathogenic *Theileria* sporozoites is directly related to disease severity in ruminants,[129,175] some *Theileria* species and strains are more virulent than others, for a variety of reasons that are not yet understood.[102] For example, in cattle, buffalo-derived *T. parva* causes Corridor disease, a more acutely fatal disease than cattle-derived *T. parva*, which causes ECF and Zimbabwe theileriosis (January disease).[93–95,102]

Vector Factors

Numerous tick factors influence the transmission success of theilerial parasites in the field.[129,174] Some tick species and strains are better vectors of pathogenic theileriosis than others.[129,131] While a wide variety of hard-bodied ticks (family Ixodidae) have been identified as vectors of diverse theilerial species,[102,129] little is known about the tick vectors of *Theileria spp.* in African wild ungulates. Specific tick genera that have been identified as vectors of *Theileria spp.* in domestic ruminants in eastern, central, and southern Africa are *Rhipicephalus, Hyalomma, Amblyomma*, and *Haemaphysalis*.[94,129] *Rhipicephalus appendiculatus* (the brown ear tick) is the most researched of the vectors because it transmits *T. parva*, the cause of ECF and Corridor disease, both deadly diseases of cattle in Africa.[126,129]

Rhipicephalus, Hyalomma, and *Amblyomma* tick species have also been identified on diverse wild African ungulates, particularly in southern Africa.[68,129,140,186] Of these, *R. appendiculatus* and *R. evertsi evertsi* (the red-legged tick), both of which have a wide host range and distribution in Africa,[135] as well as *R. pulchellus* (the zebra tick, which is largely confined to East Africa[135]), have been implicated as vectors of benign and pathogenic theileriosis in several wild artiodactyl species (Table 1). These host species include the African buffalo,[9,25,24,53,98,123] eland,[21,57,58,61,104,199] impala (*Aepyceros melampus*),[73] roan,[173,175,193,194] sable,[194] and tsessebe.[194] However, transmission of *Theileria* parasites by these *Rhipicephalus spp.* has been convincingly established only in African buffalo,[25,53,98,123] eland,[21,57,58,61,104,199] and roan antelope.[175]

Theileria species are not transmitted transovarially from infected adult ticks to their offspring.[129] Thus, to prevent elimination of theilerial parasites from a tick population, tick vectors need to be in constant contact with a source of parasites to transmit *Theileria*.[129,173] The source of parasites can be acutely sick, recently recovered, or persistent carrier hosts.[94,129,173]

Adult ticks transmit many more *Theileria* sporozoites than nymphs,[7,129] and a higher infection dose of sporozoites results in more severe disease in domestic and wild artiodactyls.[129,175] Thus, the occurrence of pathogenic theileriosis generally depends on the activity of adult ticks. In southern Africa, adult ticks seek out their vertebrate hosts in the warm, wet summer months (December to March/April).[129] As a result, most cases of theileriosis occur during late summer (Table 2).[173] However, cases of pathogenic theileriosis occur during other times of the year, especially in consistently warmer areas.

Host Factors

Diverse mammalian host factors are important determinants of disease severity.[90,94,129] These factors include age, immunity, genetic susceptibility, and stress. Young ruminants are generally more susceptible than older animals to developing pathogenic theileriosis,[94,173] although transplacental transmission of infection is rare in domestic ruminants and reported but not proven in wild artiodactyls.[89,90,173,175,194] Colostral antibodies are not generally considered to confer protective immunity against *Theileria spp.* to young ruminants.[90,173] Recently, however, it was shown that colostral transfer of maternal leukocytes including CD8+ cytotoxic T lymphocytes does occur.[182] This should confer a degree of protection to neonates, albeit for an unknown period. Evidence suggests that *T. parva* and, to an extent, *T. annulata* infections in cattle and *T. lestoquardi* infections in sheep (the cause of malignant ovine theileriosis) are largely curbed by an effective cell-mediated immune response.[45,48,50,110,116,154,178,185] Particularly in *T. parva* infections, protective immunity is principally mediated by parasite-specific major histocompatibility complex class I–restricted, CD8+ cytotoxic T lymphocyte–mediated killing of infected leukocytes.[45,110,154,185] Schizont-infected leukocytes also stimulate a parasite-specific major histocompatibility complex class II–restricted CD4+ T lymphocyte response.[45,110,154]

In some antelope species (eg, West African/Western roan antelope [*Hippotragus equinus koba*]), hypochromic poikilocytic anemia of neonates, which is thought to develop due to delayed or asynchronous perinatal switching over from fetal to adult hemoglobin, may predispose the young of these antelope species to pathogenic theileriosis.[139,173] It has been proposed that this physiologic anemia contributes to the motionlessness of Hippotragini calves during the early neonatal period, when they are sequestered from the rest of the herd for 2 to 4 weeks in tall grass or brush.[139,173] However, whether it does predispose the calves of some species to pathogenic theileriosis requires further investigation.

A geographically separated host population may be naïve to parasites that infect the host in another area. For example, Western roan calves in South Africa are more susceptible to lethal theileriosis (*Theileria sp.* [sable]) than other roan subspecies (eg, *Hippotragus equinus equinus*), because *H. e. equinus* probably evolved with *Theileria sp.* (sable) in eastern and southern Africa.[173] Similarly, translocated animals may develop disease signs due to exposure to novel *Theileria*

species or strains at the new location.[28,127,128] Furthermore, *Theileria* epidemics tend to occur when asymptomatic carrier hosts that are persistently infected with *Theileria* parasites are moved to areas previously uninfected with those particular parasites but where suitable vectors are available to initiate a transmission cycle.[94,173]

A number of stressors also increase the susceptibility of ruminants to pathogenic theileriosis.[54,142,194] Stress due to malnutrition,[173,194] translocation,[28,127,128] and high endo- and/or ectoparasite burdens[173] can also influence the outcome of theilerial infections due to altered immune function and response to infection.[67,105]

Environmental Factors

Climatic, sociodemographic, economic, and political factors play a role in disease severity, prevalence, and diversity.[46] ECF, bovine tropical theileriosis, and malignant ovine theileriosis in domestic livestock occur in specific geographic regions related to the distribution of suitable tick vectors.[89,90,94] Tick distribution depends on climate (temperature and rainfall), suitable vegetation, and host availability.[94,134] Changes in temperature and rainfall, as well as human population expansion, with associated changes in land use can affect the geographic distribution, density, and diversity of tick vectors and their vertebrate hosts.[28,41,135]

Tick habitats are also affected by livestock management systems, including methods of grazing management and tick vector control. For instance, overstocking results in social stress, increased tick burdens, and, occasionally, ensuing tick-induced immunosuppression.[44,79,173] The balance among tick, *Theileria*, and host can also be disturbed by failure to correctly rotate acaricide formulations and the application of incorrectly diluted formulations, resulting in the evolution of multiacaricide-resistant ticks.[190]

The recent explosive growth in the wildlife trade has significantly influenced land utilization, bringing wild and domestic animals into increasingly closer proximity.[41] This has facilitated the transmission of ticks and tick-borne pathogens, including *Theileria spp.*, between domestic and wild ungulate species, threatening the health of both.[13,41] Furthermore, political instability may lead to erratic implementation of tick-borne disease control measures and increased illegal movement of ruminants, their hemoparasites, and ticks.[94] This could result in outbreaks of clinical disease and fatalities in susceptible ungulates.[28]

Apart from limited data on tick vector identification in roan and other wild artiodactyls, little is known about the epidemiology of pathogenic theileriosis in wild antelope.[28,174] As a result, most existing strategies for limiting clinical disease in susceptible wild artiodactyls rely on anecdotal evidence and are unsubstantiated.

Hematology and Cytology

Information on the hematology and tissue cytology findings associated with pathogenic or transformative theileriosis in

Table 3. Hematology and Blood Smear Findings in African Wild Artiodactyls With Pathogenic Theileriosis.

Parameters	Artiodactyl Species[a]
Leukocyte fraction	
Leukocytosis	EI eland (*Tragelaphus oryx*),[b,58] greater kudu (*Tragelaphus strepsiceros*),[123] EI and NI Western roan (*Hippotragus equinus koba*)[174]
Leukopenia	EI African buffalo (*Syncerus caffer*),[b,9] EI eland[b,57]
No significant change in leukocyte count	African buffalo,[b,9] EI eland[54,57]
Erythrocyte fraction	
Regenerative anemia[c]	Eland,[54] giraffe (*Giraffa camelopardalis*),[108] greater kudu,[123] Western roan[173,174]
Decreased red cell count	EI Eland,[54,57] greater kudu[123]
Decreased packed cell volume	EI Eland[54,57]
Decreased hemoglobin concentration	EI Eland[54,57]
Percentage piroplasm parasitemia	Up to 3% EI African buffalo,[25] 15% EI African buffalo,[b,86] 10% African buffalo,[b,205] 70% greater kudu,[123] 5% gray/common duiker (*Sylvicapra grimmia*),[125] up to 90% eland,[21,104] up to 50% EI and NI eland,[54,57] up to 14.4% sable (*Hippotragus niger*),[193,194] 17.4% and 31.9% in 2 NI roan (*Hippotragus equinus*),[193,194] up to 70% EI and NI Western roan,[173] 30% Tsessebe (*Damaliscus lunatus*)[75]
Platelet fraction	
Thrombocytopenia	EI and NI Western roan[173]

Abbreviations: EI, experimentally infected NI, naturally infected.
[a]Unless indicated differently, infection was natural.
[b]Nonfatal infection.
[c]Erythrocyte anisocytosis, basophilic stippling, polychromasia, Howell-Jolly bodies, normoblasts, and/or reticulocytes observed in blood smears.

African wild artiodactyls is summarized in Table 3. Typical intraleukocytic theilerial schizonts (Koch blue bodies) have been reported in blood and buffy coat smears stained with Romanowsky stains from a variety of African wild ungulates with natural and experimental theileriosis.[128,174,175,181,193,194] However, there is only a single description of theilerial schizont morphology in a Giemsa-stained blood smear from a sable calf in southern Africa.[181] In this report, schizonts within host leukocytes were similar to those described in cattle with ECF[94,123] and contained variable numbers of chromatin granules/nuclei dispersed in a shared pale blue or neutral cytoplasm (Fig. 5). Similar to ECF, schizont nuclei were smaller but more numerous in the micro- versus the macroschizont stage of parasite development in sable peripheral blood leukocytes.[181]

Theilerial schizonts have been well described in organ impression smears and cultured leukocytes (Fig. 6) sourced from peripheral blood and lymph node biopsy samples from
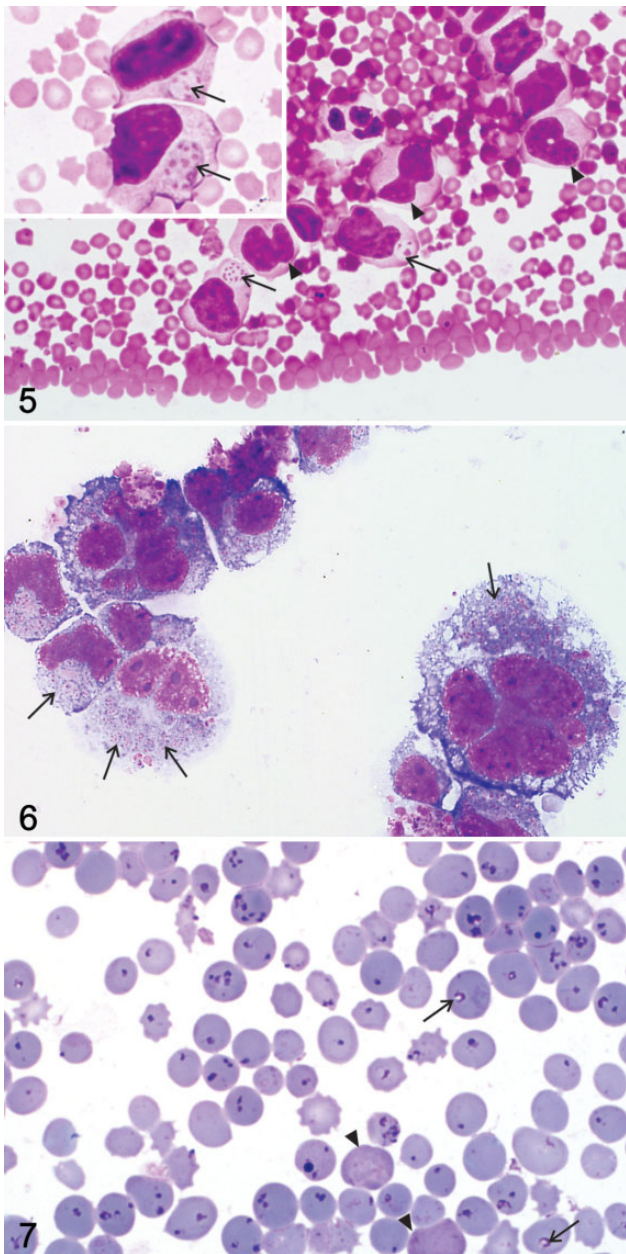
Figures 5–7. Pathogenic *Theileria sp.* (sable) infection, blood and tissue culture smears, Western roan antelope (*Hippotragus equinus koba*). **Figure 5.** Atypical mononuclear leukocytes in a peripheral blood smear have indented hyperchromatic nuclei (arrowheads), multiple nucleoli, and moderate homogeneous pale basophilic cytoplasm. Some leukocytes contain parasitic schizonts characterized by variable numbers of pleomorphic nuclei dispersed within a shared neutral or pale blue cytoplasm (arrows). Inset: Higher magnification of schizont-infected (arrows) leukocytes from a different area of the same blood smear. Diff-Quik. **Figure 6.** Atypical multinucleated giant macrophage-like cells in tissue culture, with multiple variably prominent nucleoli and moderate to ample basophilic cytoplasm with occasional vacuoles. *Theileria* parasites are clustered and dispersed within the cytoplasm of these cells (arrows). Giemsa. **Figure 7.** The majority of erythrocytes in a peripheral blood smear contain pleomorphic intraerythrocytic piroplasms with signet ring (arrows), bacillary, anaplasmoid, and comma-shaped forms. Multiple piroplasms are present in some erythrocytes. Reticulocytes are also evident (arrowheads). Diff-Quik.

diverse healthy and sick artiodactyls.[**] The reason is that, before the advent of molecular diagnostic techniques, microscopic schizont morphology and infected leukocyte phenotype (without immunophenotypic characterization) formed the basis for differentiation between *Theileria* and *Cytauxzoon* genera and among *Theileria* species.[21,23,75,108,123,125,193,196] The reported average schizont size, number of nuclei per schizont, size of schizont nuclei, and number of intraleukocytic schizonts vary considerably among African wild artiodactyl species with pathogenic theileriosis, but extra- and intraleukocytic schizonts in tissue smears were likened to the schizonts observed in cattle with *T. parva* (ECF).[††] Reasons that have been proposed for the wide variation in domestic and wild artiodactyls include differences in schizont maturity,[123,125] stage of disease (with fewer parasites visible in acutely fatal infections),[93] and differences in theilerial parasite pathogenicity.[93]

The identity of the schizont-transformed leukocytes in blood, buffy coat, and lymph node smears from wild ungulates with pathogenic theileriosis has been the subject of debate for decades. These transformed leukocytes have been variably referred to as lymphoblasts, lymphoblastoid cells, or lymphoid cells in African buffalo,[22,197] eland,[54,57,58] and roan antelope (Fig. 5).[128,173–175] A monocyte-macrophage derivation for these leukocytes was also suggested in the gray/common duiker,[123,125,128] greater kudu,[123] and roan and sable calves.[174,175,193,194] In addition to parasitized mononuclear macrophage-like host cells, infected multinucleated giant macrophage-like cells with ≥17 nuclei were reported in tissue and/or organ smears from a gray/common duiker[123,125] and a greater kudu in South Africa.[123] In roan calves, infected and uninfected leukocytes were often highly atypical and hard to distinguish from neoplastic cells (Fig. 6).[174] They possessed irregularly indented, lobed, and, rarely, multiple nuclei, multiple prominent nucleoli, mitoses, and moderate to ample and occasionally vacuolated basophilic cytoplasm.[174] Schizont-transformed leukocytes in blood and organ smears have not yet been immunophenotyped in any African wild artiodactyl species, so their identity remains unknown.

Several studies have investigated the effects of pathogenic *Theileria* parasites on the leukocyte count in naturally and/or experimentally infected African buffalo,[9] eland,[54,57,58] greater kudu,[123] and juvenile Western roan antelope.[174] Leukocytosis, leukopenia, and normal leukocyte counts have been reported within and among selected wild ungulate species.

There is a surplus of detailed information on the morphology of theilerial piroplasms and the percentage of piroplasm-infected erythrocytes estimated in blood smears in healthy[‡‡] and sick wild African ungulates.[***] In many of these reports, the authors observed marked piroplasm pleomorphism, with

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[**]References 11, 21, 26, 54, 57, 58, 98, 99, 104, 118, 119, 123, 125, 159, 169–171, 176, 193, 194, 199, 204, 205
[††]References 11, 25, 26, 57, 99, 104, 125, 174, 175, 194
[‡‡]References 10, 15, 22, 26, 32, 59, 73, 118, 119, 181, 183
[***]References 21, 32, 54, 57–59, 75, 108, 123, 125, 128, 173, 175, 181, 193, 194

shapes varying from round or signet ring shaped to elongated/ bacillary, anaplasmoid, comma shaped, and oval/ovoid as well as paired and tetrad/Maltese cross forms (Fig. 7). Theilerial piroplasms occasionally possessed a crescentic or cap-shaped dense nucleus on the margin of round piroplasms and at the wider extremity of ovoid to pear-shaped/pyriform parasites; the cytoplasm was not often visible in the bacillary, comma-shaped, or anaplasmoid forms.[32,35,123,183] In addition, theilerial piroplasms have been described as small, generally measuring 0.5 to 2.5 μm in width or diameter (depending on their shape) and up to 2.5 μm in length.[75,118,123,183] Between 1 and 12 piroplasms were observed within a single erythrocyte in diverse wild artiodactyl species, with 1 to 4 (tetrads) being most commonly observed.[21,75,118,123,125]

Percentages of piroplasm-infected erythrocytes that have been reported in sick or fatally infected wild artiodactyls are highly variable within and among species. The erythrocyte infection rate was often <50% but was occasionally as high as 90% or <3%. The level of piroplasm infection may not be related to the severity of disease in wild antelope. In cattle with acutely fatal Corridor disease, parasites are often scarce or even absent in blood and tissue smears.[8,22,26,93,124]

Regenerative anemia of variable severity, largely based on the observation of mucous membrane pallor and evidence of a regenerative erythrocyte response in blood smears,[123] has been reported in eland,[54] giraffe,[108] greater kudu,[123] and Western roan antelope[173,174] with pathogenic theileriosis. In experimentally infected eland[54,57] and a naturally infected greater kudu,[123] parameters such as red cell count, packed cell volume, and hemoglobin concentration have been found to decrease after Theileria infection. However, as the algorithm used to separate erythrocyte and platelet populations on cell counters and analyzers may not have been appropriate for the species, the reported red cell counts may not be accurate.

Thrombocytopenia[173] and prolonged bleeding times[128] have also been reported in Western roan calves with terminal Theileria sp. (sable) infection. However, these findings are not validated in roan antelope, and the effects of pathogenic theileriosis on platelets have not been investigated in other wild antelope species. Although hematologic reference values exist for a variety of wild artiodactyl species in captivity and in the wild,[39,149–152] testing methods need to be standardized to allow for accurate inference over diverse species.[165]

Macroscopic Pathology

Gross lesions reported in wild artiodactyls with pathogenic theileriosis are very similar to those in domestic livestock with ECF (T. parva), bovine tropical theileriosis (T. annulata), and malignant ovine theileriosis (T. lestoquardi).[19,35,72,82,89,90,94] Tissue lesions are predominantly the result of focal to diffuse infiltration with hyperproliferative, schizont-transformed leukocytes and associated parenchymal necrosis, hemorrhage, and inflammation (see Histopathology section for details). Table 4 lists the salient macroscopic lesions reported in diverse wild ungulate species.

Anemia and icterus were commonly observed (Fig. 8), although severity was variable within (eland[21,54] and roan[174,193]) and among species.[†††] Severe carcass congestion was reported only in a gray/common duiker with fatal theileriosis.[123,125]

Hemoglobinuria, due to intravascular hemolysis, and bilirubinuria are features of pathogenic theileriosis in domestic livestock, particularly bovine tropical theileriosis and malignant ovine theileriosis.[70,77,89,90] Hemoglobinuria is not a common feature of ECF in cattle[72,94] but was observed in eland,[21,104] giraffe,[108] and roan antelope[193] with fatal pathogenic theileriosis. Bilirubinuria in the absence of hemoglobinuria also occurred in Western roan calves with fatal Theileria sp. (sable) infection.[174] Thus, significant intravascular hemolysis and hemoglobinuria are not consistent features of pathogenic theileriosis in ruminants, although this requires further investigation in African wild artiodactyls.

Splenomegaly and widespread lymphadenomegaly have been frequently reported across diverse species, mainly due to focal/nodular or diffuse leukocyte proliferation (Figs. 9, 10).[‡‡‡] Lymphadenomegaly due to edema was described in a tsessebe,[75] and widespread lymph node edema in the absence of lymphadenomegaly was observed in a giraffe.[108]

Prominent lung edema was another frequent but inconsistent[54] observation in diverse species (Fig. 9).[21,54,57,123,125,128,173,175] Diffusely hyperemic, mottled lungs with increased consistency due to widespread atypical leukocyte infiltration and associated hemorrhage and inflammation, typical for interstitial pneumonia at the gross pathology level, has been reported only in a tsessebe.[75]

Similar foci of leukoproliferation and hemorrhage in the kidneys, with the macroscopic appearance of interstitial nephritis, which were initially and incorrectly described as renal infarcts in early reports describing the gross appearance of the kidneys in fatal ECF in cattle,[51,81,179] were also reported in eland.[99,123] Affected wild ungulates also commonly showed hepatomegaly due to widespread infiltration of schizont-transformed leukocytes with associated hepatocyte injury and inflammation (Fig. 11).[75,108,123,125,174] Nodular leukocyte infiltration was associated with a multinodular liver ("tumor hepatis") in an eland and a gray/common duiker.[123]

Diarrhea was a common finding in eland with pathogenic theileriosis.[11,54,123] It has been typically associated with marked thickening of the abomasum and small intestine due to extensive leukoproliferation with secondary erosive to ulcerative gastroenteritis and mucosal barrier disruption.[54] Multifocal abomasal erosions were also described in 1 of 2 gray/common duikers that succumbed to pathogenic theileriosis.[123]

Serosanguinous effusions into body cavities (pericardium, thorax, and abdomen) were reported in multiple wild ungulate

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[†††]References 21, 54, 75, 108, 123, 128, 173, 174, 193, 194
[‡‡‡]References 10, 21, 54, 57, 75, 104, 123, 125, 128, 173, 174

Table 4. Macroscopic Pathology Observed in African Wild Artiodactyls With Fatal Pathogenic Theileriosis.[a]

Parameters	Artiodactyl Species[b]
Anemia	EI and NI eland (*Tragelaphus oryxx*),[21,54] giraffe (*Giraffa camelopardalis*),[108] greater kudu (*Tragelaphus strepsiceros*),[123] roan (*Hippotragus equinus*),[193,194] EI and NI Western roan (*Hippotragus equinus koba*),[128,173,174] sable (*Hippotragus niger*),[128] tsessebe (*Damaliscus lunatus*)[75]
Icterus	Eland,[21,104] greater kudu,[123] roan,[193] EI and NI Western roan,[128,173,174] sable[128]
Congestion	Gray/common duiker (*Sylvicapra grimmia*)[125]
Poor body condition	EI and NI eland,[11,54,57] greater kudu,[123] gray/common duiker,[123,125] EI Western roan[175]
Normal to good body condition	EI and NI eland,[54] giraffe,[108] gray/common duiker,[128] roan,[193] Western roan,[173] tsessebe[75]
Petechiae and ecchymoses	Eland,[21,54] giraffe,[108] gray/common duiker,[123,128] EI and NI Western roan,[128,173,174] sable,[128] tsessebe[75]
Suffusive hemorrhages	Giraffe,[108] EI Western roan[174]
Fluid effusions into body cavities (pericardium, thorax and/or abdomen)	EI and NI eland,[54,57] giraffe,[108] greater kudu,[123] gray/common duiker,[123,125] roan,[194] EI and NI Western roan,[173,174] tsessebe[75]
Anasarca	Eland,[21] greater kudu[123]
Brain edema	Sable[128]
Lung edema	EI and NI eland,[21,54,57] greater kudu,[123] gray/common duiker,[123,125,128] EI and NI Western roan,[128,173,174] sable[128]
Interstitial pneumonia	Tsessebe[75]
Lymph node edema	Giraffe,[108] tsessebe[75]
Lymphadenomegaly due to leukocyte hyperplasia	African buffalo (*Syncerus caffer*),[10] EI and NI eland,[21,54,57,104] greater kudu,[123] gray/common duiker,[128] EI and NI Western roan,[128,173,174] tsessebe[75]
Splenomegaly due to red and/or white pulp hyperplasia	African buffalo,[10] EI and NI eland,[54,57] greater kudu,[123] gray/common duiker,[123,125,128] EI and NI Western roan,[128,173,174] sable,[128] tsessebe[75]
Hepatomegaly with or without cholestasis	Eland,[99] giraffe,[108] greater kudu,[123] gray/common duiker,[125] EI Western roan,[174] sable,[128] tsessebe[75]
Multinodular liver	Eland,[123] gray/common duiker[123]
Ulcerative abomasitis and enteritis	EI and NI eland,[21,54] gray/common duiker[123,125,128]
Enteric hemorrhage	EI Western roan[174]
Diarrhea	Eland[11,54,123]
Interstitial nephritis	Eland[99,123]
Hemoglobinuria	Eland,[21,104] giraffe,[108] roan[193]
Bilirubinuria	EI Western roan[174]

Abbreviations: EI, experimentally infected; NI, naturally infected.
[a]Macroscopic lesions are the result of focal to diffuse tissue infiltration with hyperproliferative schizont-transformed leukocytes and associated parenchymal necrosis, hemorrhage, and inflammation.
[b]For all cases where EI/NI is not specified, natural infection is implied.

species.**** Anasarca has been reported in eland[21] and a greater kudu.[123]

Petechiae and ecchymoses were commonly observed on serosal and mucosal surfaces and the subcutis and in selected organs, including peripheral and visceral lymph nodes, liver (Fig. 11), heart (Fig. 12), lungs, kidneys, urinary bladder, gall bladder, gastrointestinal tract, and skeletal muscles.[21,54,75,108,123,128,173,174] Serosal suffusive hemorrhages have been observed in giraffe[108] and Western roan carcasses[174]; diffuse enteric hemorrhage was also reported in the latter.[174]

In wild artiodactyls with pathogenic theileriosis that lacked other diseases, poor body condition was ascribed to *Theileria*-associated anorexia and dehydration (Western roan[174]), diarrhea due to infiltration of the intestinal wall with atypical leukocytes (eland[54]), and chronic infection (eland[54] and Western roan[174]). However, in a greater kudu and gray/common duiker that died of schizont-"transforming" theileriosis, other possible causes of weight loss, including the stress of recent translocation, climate (eg, drought), and feed scarcity, were not discussed.[123,125]

Poor body condition might predispose wild artiodactyls to severe tick infestation and therefore increase their likelihood of contracting pathogenic theileriosis,[113,194] although this assertion is unproven. Wild ungulates in good and poor body condition that succumbed to pathogenic theileriosis were reportedly variably tick infested.[54,173] Moreover, the number of ticks observed at necropsy may not be a true reflection of the premortal tick burden, because ticks start to drop off carcasses soon after death.[173]

Detailed macroscopic descriptions are needed in wild artiodactyls that die due to schizont-"transforming" theileriosis. In particular, the association between pathogenic theileriosis and poor body condition requires in-depth investigation across diverse species. ECF, malignant ovine theileriosis, and, to a

****References 54, 57, 75, 108, 123, 125, 173, 174, 194
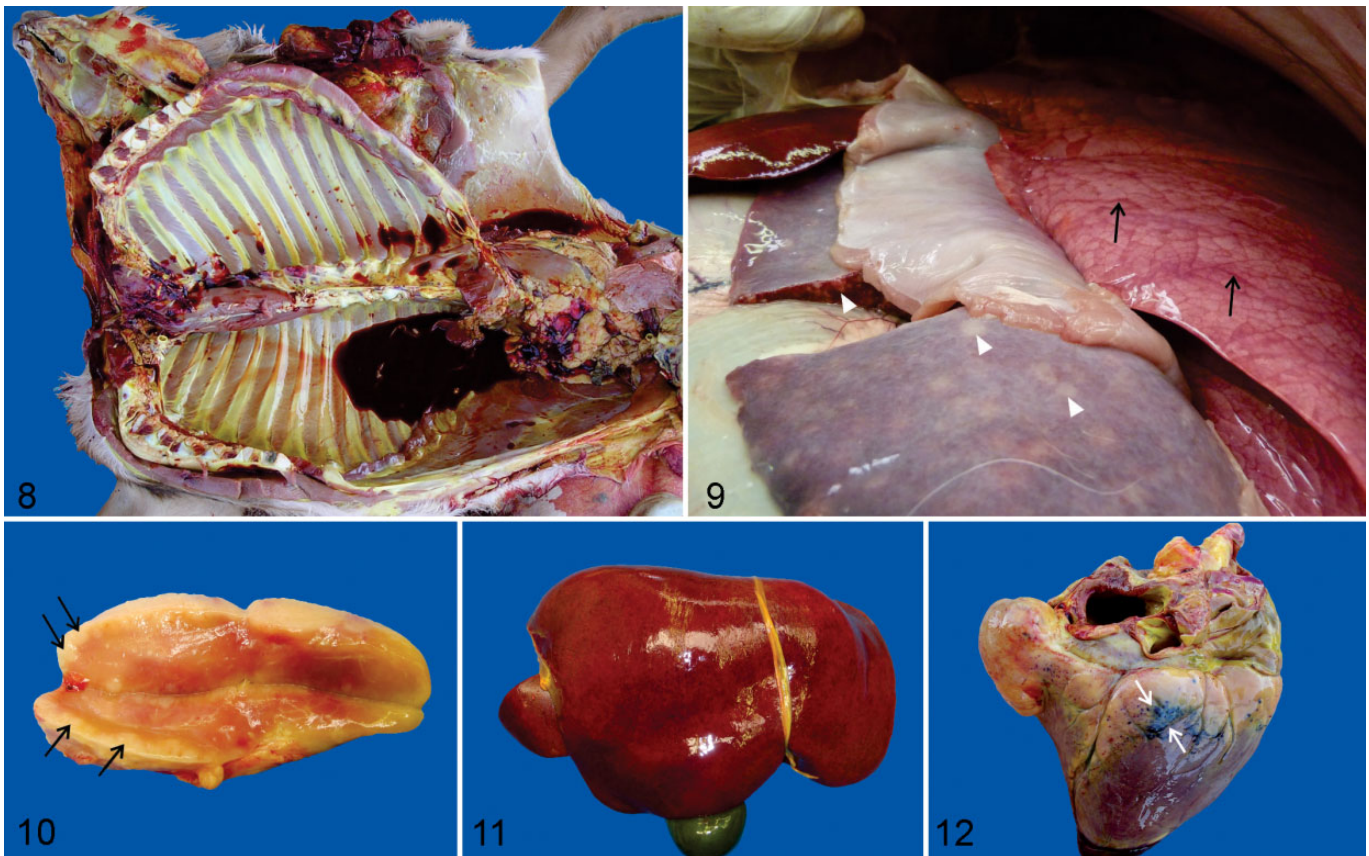
Figures 8–12. Pathogenic *Theileria sp.* (sable) infection. **Figure 8.** Western roan antelope (*Hippotragus equinus koba*). Icterus, anemia, and unclotted blood. **Figure 9.** Spleen and lung, waterbuck (*Kobus ellipsiprymnus*). Splenomegaly due to multinodular foci of host leukocyte proliferation (arrowheads). Note the interstitial lung edema leading to widening of the interlobular septa (arrows). **Figure 10.** Lymph node, sable antelope (*Hippotragus niger*). Lymphadenomegaly due to multinodular proliferation of host leukocytes in the cortex (arrows). **Figure 11.** Liver, sable antelope (*H. niger*). Hepatomegaly due to widespread infiltration of atypical leukocytes and reversible and irreversible hepatocyte injury. The liver is also diffusely congested with cholestasis and widespread petechiation. **Figure 12.** Heart, Western roan antelope (*H. equinus koba*). There are epicardial petechiae and ecchymoses (arrows).

lesser extent, bovine tropical theileriosis have all been associated with emaciation and, in rare cases, also chronic infection or a state of partial recovery from initial infection.[89,90,94] These cases are characterized by emaciation, anemia, generalized edema, lymphoid atrophy, and secondary infections due to immunosuppression.[71,89,94] Currently it is not known whether a chronic debilitating form of pathogenic theileriosis exists in wild artiodactyls.

Histopathology

The histopathology of pathogenic theileriosis is well described for ECF in cattle[6,35,45,82,94,123] and bovine cerebral theileriosis/turning sickness,[14,33,155,187] but there are comparatively few descriptions of the histopathology of bovine tropical theileriosis[19,90] and malignant ovine theileriosis[71] in domestic livestock. Similarly, comprehensive histopathologic descriptions of pathogenic theileriosis are scarce in African wild artiodactyls. Lesions have been best described in the African buffalo,[10] eland,[21,54,104] giraffe,[108] gray/common duiker,[123,125] and

tsessebe,[75] although, in most cases, only a single animal of each species was necropsied.[21,75,104,108,125]

Details of the histologic lesions described in transforming theileriosis across diverse African wild artiodactyl species are listed in Table 5. The following is a summary of key lesions reported. The pivotal lesion is, similar to that in cattle with ECF, multifocal to diffuse infiltrates of target cells in multiple organs.[††††] Common secondary lesions include congestion, multifocal disseminated hemorrhages, foci of parenchymal necrosis, and inflammation. For the purpose of this review, target cells refer to host cells that may or may not be schizont infected but are induced to undergo pseudo-neoplastic transformation (as evidenced by cellular atypia), as well as hyperproliferation and widespread dissemination.[31] Target cell phenotype and location in various species are summarized in Table 6. Similar to ECF, widespread lysis of initially proliferative target cells has been reported.[54,75,108,125]

[††††]References 10, 21, 26, 54, 75, 108, 123, 125, 174, 193, 194

Table 5. Significant Histopathologic Findings in African Wild Artiodactyls With Fatal Pathogenic Theileriosis.

LN	Spleen	Lung	Liver	Kidney	Heart	Skeletal Muscle	GIT	Adrenal	Brain	Artiodactyl Species[a]
										Target cell infiltration
×	×	×	×	×	×	−	−	−	−	African buffalo (*Syncerus caffer*)[10]
×	×	×	×	×	×	−	×	×	×	El and NI eland (*Tragelaphus oryx*)[21,54,104]
−	×	×	×	×	−	−	×	−	−	Giraffe (*Giraffa camelopardalis*)[26,108]
−	×	×	×	×	×	−	×	−	×	Gray/common duiker (*Sylvicapra grimmia*)[123,125]
−	−	−	−	−	×	−	−	×	−	Roan (*Hippotragus equinus*)[193,194]
×	×	×	×	×	−	−	−	×	−	El and NI Western roan (*Hippotragus e. koba*)[173,174]
−	×	×	×	×	×	×	−	−	−	Tsessebe (*Damaliscus lunatus*)[75]
										Schizonts
×	−	−	−	−	×	−	−	−	−	African buffalo[10]
×	×	×	×	×	×	−	×	×	×	El and NI eland[8,21,54,57,61,104]
−	×	×	×	−	−	−	×	−	−	Giraffe[108]
−	×	×	×	×	×	−	×	−	×	Gray/common duiker[123,125]
−	−	−	−	−	×	−	−	×	−	Roan[193,194]
×	×	×	×	×	−	−	−	×	−	El and NI Western roan[173,174]
−	×	×	×	×	−	−	−	−	−	Tsessebe[75]
										Disseminated foci of necrosis
−	−	−	−	−	×	×	−	−	−	African buffalo[10]
×	×	×	×	−	−	−	×	×	×	El and NI eland[21,54,104]
−	×	−	×	−	−	−	×	−	−	Giraffe[108]
−	−	−	×	−	−	−	−	−	−	Greater kudu[123]
−	−	−	−	×	×	−	−	−	×	Gray/common duiker[123,125]
−	×	−	×	×	×	×	−	−	−	Tsessebe[75]
										Congestion
−	−	×	−	−	−	−	×	−	×	El and NI eland[54]
−	−	×	×	−	−	−	−	−	−	Giraffe[108]
×	−	×	×	×	−	×	−	×	×	Greater kudu[123]
−	×	×	×	×	×	−	×	−	×	Gray/common duiker[123,125]
−	−	×	−	−	−	−	−	−	−	El Western roan[174]
−	×	×	×	×	−	−	−	−	−	Tsessebe[75]
										Multifocal disseminated hemorrhages
−	−	×	−	−	−	−	−	−	×	El and NI eland,[54] gray/common duiker[123,125]
−	−	×	−	−	−	−	×	−	−	Giraffe[108]
−	−	−	−	−	−	−	−	×	−	Greater kudu[123]
−	−	×	−	−	−	−	−	−	−	El Western roan[174]
−	−	−	×	×	×	×	−	−	−	Tsessebe[75]
										Edema
−	−	×	−	−	−	−	×	−	−	El and NI eland[54]
−	−	×	−	−	−	−	−	−	−	Giraffe,[108] gray/common duiker,[123,125] El Western roan[174]
×	−	−	−	−	−	−	−	−	−	Greater kudu[123]
										Fibrinous exudate
−	−	×	−	−	−	−	−	−	−	Eland,[54] giraffe,[108] greater kudu,[123] gray/common duiker,[123,125] El Western roan[174]
										Lymphocytolysis and/or atrophy of normal lymphoid tissue
×	×	−	−	−	−	−	−	−	−	El and NI eland[54]
−	×	−	−	−	−	−	−	−	−	Tsessebe[75]
										Thromboemboli
×	−	×	×	−	−	−	−	×	×	El and NI eland[54]
−	−	×	×	−	−	−	×	−	×	Gray/common duiker[123,125]
										Vasculitis
−	×	−	×	−	×	−	×	−	−	Gray/common duiker[123,125]

Abbreviations: EI, experimentally infected; GIT, gastrointestinal tract (abomasum and intestine); LN, lymph nodes; MPS, mononuclear phagocytic system; NI, naturally infected.
[a] For all cases where EI/NI is not specified, natural infection is implied.

As in tissue smears, large mononuclear target cells that have been described as lymphoid and/or macrophage like in histologic sections were characterized by round to bean-shaped/ reniform eccentrically situated nuclei, multiple prominent nucleoli, and ample homogenous to vacuolated amphiphilic cytoplasm (Fig. 13).[54,75] Multinucleated giant cells (thought

Table 6. Target Cell Phenotype and Location and Parasitic Schizont Location in Histologic Sections From African Wild Artiodactyls With Fatal Pathogenic Theileriosis.

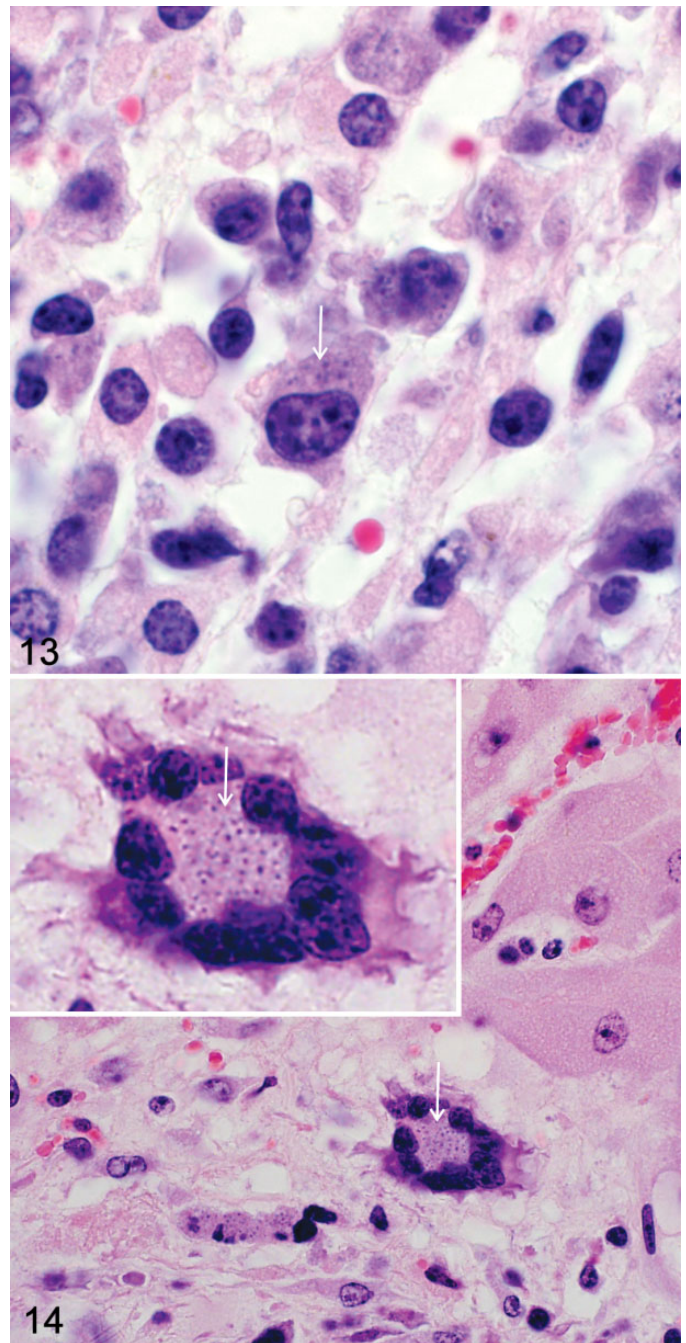| Parameter	Artiodactyl Species[a]
Target cells[b]	
Mononuclear leukocytes (lymphocytes and/or macrophages)	African buffalo (*Syncerus caffer*),[10] EI and NI eland (*Tragelaphus oryx*),[54] giraffe (*Giraffa camelopardalis*),[108] greater kudu (*Tragelaphus strepsiceros*),[8,123] gray/common duiker (*Sylvicapra grimmia*),[8,125,128] roan (*Hippotragus equinus*),[193,194] EI and NI Western roan (*Hippotragus e. koba*),[173,174] tsessebe (*Damaliscus lunatus*)[75]
Multinucleated/syncytial giant cells	Eland,[8,21,104] giraffe (scarce),[108] gray/common duiker,[8,123,125] tsessebe (scarce)[75]
Tissue-resident macrophages	African buffalo,[10] giraffe,[108] tsessebe[75]
Hepatocytes	Eland,[8,21,104] giraffe[108]
Target cell location within organs	
Intravascular	EI and NI eland,[8,21,54] gray/common duiker,[8,123,125] EI Western roan,[174] tsessebe[75]
Within vascular walls	African buffalo,[10] gray/common duiker[125]
Perivascular/interstitial	African buffalo,[10] EI and NI eland,[54] giraffe,[108] gray/common duiker,[123,125] EI Western roan,[174] tsessebe[75]
Schizont/merozoite location	
Intravascular	EI and NI eland,[8,21,104] giraffe,[108] gray/common duiker[123,125]
Perivascular/interstitial	African buffalo,[10] EI and NI eland,[21,104] giraffe[108]

Abbreviations: EI, experimentally infected; NI, naturally infected.
[a] For all cases where EI/NI is not specified, natural infection is implied.
[b] Target cells refer to host cells that may or may not be schizont infected but are induced by parasitic schizonts to undergo pseudo-neoplastic transformation (as evidenced by cellular atypia), as well as hyperproliferation and widespread dissemination.[31]

Figures 13 and 14. Pathogenic *Theileria sp.* (sable) infection, waterbuck (*Kobus ellipsiprymnus*). Hematoxylin and eosin. **Figure 13.** Adrenal gland. Intrasinusoidal mononuclear target cell characterized by an eccentrically situated bean-shaped nucleus, multiple nucleoli, and ample amphophilic cytoplasm. Basophilic parasitic nuclei (arrow) are dispersed in the cytoplasm adjacent to the host cell nuclear indentation. **Figure 14.** Liver. Multinucleated giant target cell with multiple prominent nucleoli in the interstitium of a portal tract. Basophilic parasitic nuclei are located centrally (arrow). Inset: higher magnification.

to be macrophage derived) have been inconsistently reported (Fig. 14).[21,75,108,123,125] Parasitized mono- or multinucleated target cells in the liver were thought to be severely hypertrophied hepatocytes.[21,108] Tissue-resident macrophages, including alveolar macrophages, Kupffer cells, and/or splenic macrophages, were commonly identified as target cells.[10,75,108] However, to date, no immunophenotyping has been done on tissue sections to further investigate the identity of the target cells in African wild ungulates.

As has been observed in cattle with ECF,[45] target cells infiltrate diverse lymphoid and nonlymphoid organs in wild artiodactyls with pathogenic theileriosis. Affected organs include the following (from most to least commonly infiltrated): lung (Fig. 15), liver (Fig. 16), spleen (Fig. 17), kidney (Fig. 18), heart (Fig. 19), peripheral and visceral lymph nodes (Fig. 20), gastrointestinal tract (abomasum and small intestine; Fig. 21), adrenal glands (Fig. 22), brain (Fig. 23), and skeletal
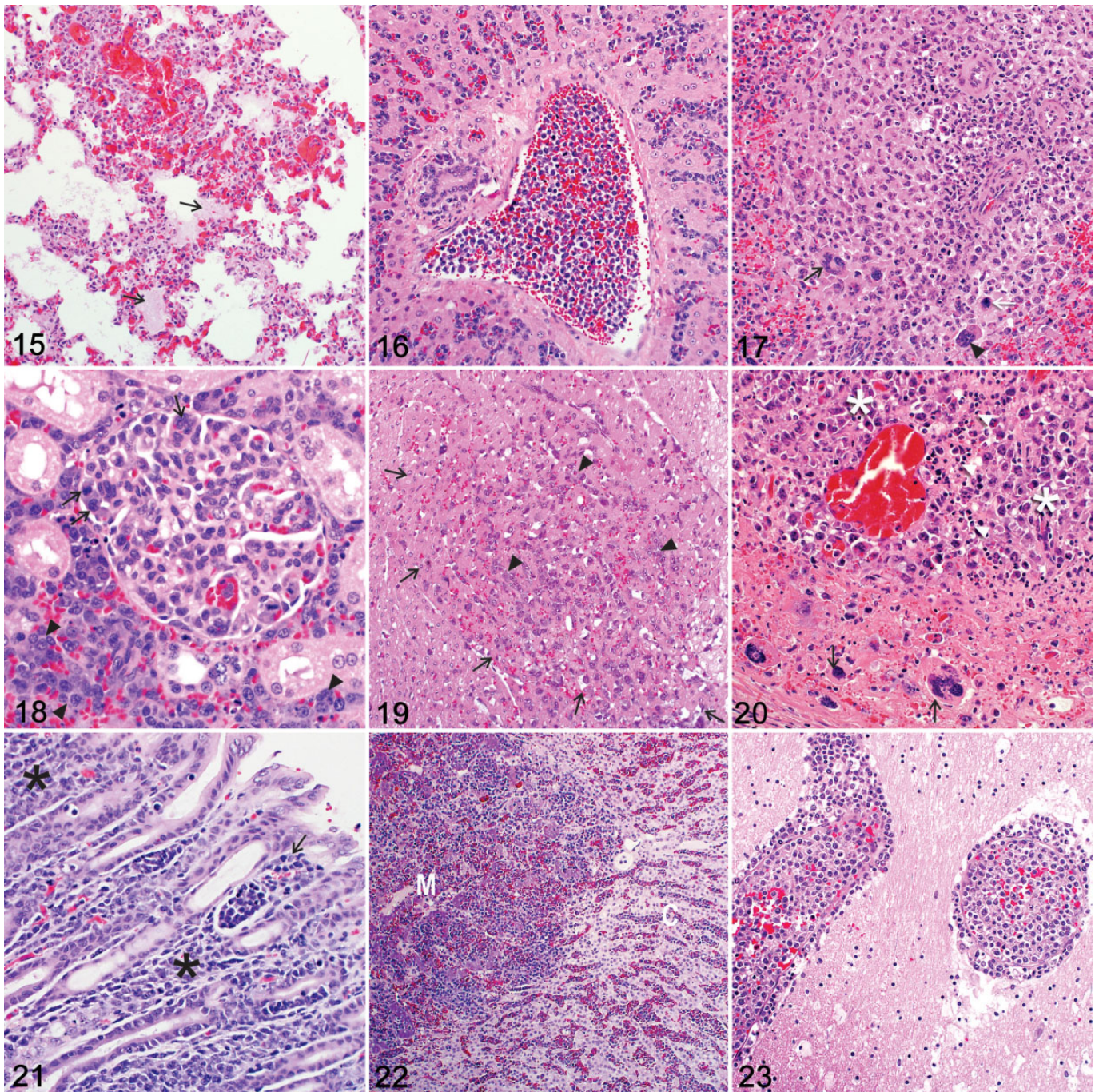
Figures 15–23. Fatal pathogenic theileriosis. Hematoxylin and eosin. **Figure 15.** Lung, tsessebe (*Damaliscus lunatus*). Congestion, hemorrhage, proteinaceous edema (arrows), and thickening of the alveolar septa due to widespread leukocyte infiltration. **Figure 16.** Liver, eland (*Tragelaphus oryx*). The portal vein and sinusoids are congested and contain abundant mononuclear leukocytes with hyperchromatic nuclei. **Figure 17.** Spleen, gray/common duiker (*Sylvicapra grimmia*). The white pulp is infiltrated by large atypical leukocytes with evidence of multinucleation (black arrow), karyomegaly (arrowhead), and mitoses (white arrow). The normal lymphoid tissue is atrophic. **Figure 18.** Kidney, eland (*T. oryx*). Diffuse congestion and plugging of glomerular (arrows) and interstitial (arrowheads) capillaries with abnormal leukocytes, characterized by irregularly indented hyperchromatic nuclei and prominent nucleoli. **Figure 19.** Heart, roan antelope (*Hippotragus equinus*). Focal area of myocardial necrosis signified by hypereosinophilic myofibers (area delineated by arrows), associated with congestion and dispersed mononuclear leukocytes (arrowheads). **Figure 20.** Lymph node, gray/common duiker (*Sylvicapra grimmia*). Fibrin, hemorrhage, nuclear debris, and bizarre giant mono- and multinucleated leukocytes (arrows) are visible in the subcapsular sinus. Proliferating leukocytes have infiltrated the cortex (asterisks), and many are necrotic (arrowheads). **Figure 21.** Abomasum, eland (*T. oryx*). Intravascular (arrow), interstitial, and intraepithelial leukocytes are present in the superficial mucosa (asterisks). **Figure 22.** Adrenal gland, eland (*T. oryx*). Diffuse congestion and widespread infiltration of leukocytes with hyperchromatic nuclei into the cortex (C) and medulla (M). **Figure 23.** Brain, eland (*T. oryx*). Blood vessels in the white matter of the cerebral cortex are distended and surrounded by parasitized and nonparasitized mononuclear leukocytes.

muscles (Table 5). Target cells and/or extracellular theilerial schizonts/merozoites have been reported within and around small- and medium-caliber arteries, arterioles, veins, capillaries, sinusoids, and/or lymphatics.[10,21,75,104,108,123,125,174] Necrotizing vasculitis was an inconsistent finding.[123,125] Vascular distension and occlusion by target leukocytes and/or fibrin, often associated with foci of parenchymal necrosis, has been described (Figs. 16, 18, 22, and 23).[54,123,125] Necrotic foci were most frequently reported in the liver, followed by the heart (Fig. 19), spleen, skeletal muscle, gastrointestinal tract, and brain.[10,21,54,75,108,123,125] Further investigation is needed to determine whether these are all due to vascular occlusion. Foci of hemorrhage and/or congestion was commonly described in the lungs (Fig. 15) and, to a lesser extent, the liver (Fig. 16), kidney, and brain.[54,75,108,123,125,174]

As might be expected, parasitic intracytoplasmic schizonts/merozoites were most frequently identified in those organs most commonly infiltrated by target cells (Table 5). Schizonts have been identified as clusters of distinct pleomorphic, darkly basophilic chromatin granules/nuclei embedded within a neutral-staining parasitic cytoplasm that may displace target cell nuclei to one side (Figs. 13, 14).[54,75,94,108] Their size, number, internal structure, occurrence, and distribution in different tissues and organs vary considerably within and among wild artiodactyl species.[‡‡‡‡]

Similar to ECF,[45] widespread pulmonary intra- and perivascular mononuclear target cell infiltrates have been described with diffuse congestion, proteinaceous edema (Fig. 15), multifocal disseminated hemorrhages, and multifocal alveolar septal necrosis associated with thrombi.[10,54,75,108,123,125,174] Parasitic schizonts were observed in alveolar macrophages[75] and multinucleated target cells.[21,75,123,125] Reference was made to interstitial pneumonia in a tsessebe[75] and mild pneumonitis in a giraffe.[108] Pulmonary histopathology in the tsessebe was characterized by diffuse congestion and widespread lymphocyte and macrophage infiltration, although there was no mention of leukocyte atypia. Pneumonitis in the giraffe referred specifically to multifocal disseminated foci of interstitial and intra-alveolar fibrin exudation and hemorrhage. Infiltrating parasitized and nonparasitized leukocytes were observed only in a focal area of giraffe lung.

In the liver, target cells were observed in sinusoids and portal and hepatic veins as well as in the perivascular interstitium of the portal tracts (Fig. 16) frequently associated with intra- and extracellular theilerial schizonts.[54,75,108,123,125,174] Parasitized Kupffer cells occasionally surrounded foci of hepatocyte necrosis and hemorrhage.[75,108] Parasitized multinucleated giant cells of indeterminate origin[21,75,108,123,125] were rarely and inconsistently associated with necrotizing vasculitis.[123,125]

Target cells have been described predominantly within the splenic red pulp, but the white pulp was also occasionally infiltrated (Fig. 17).[10,54,75,108,123,125,174] Foci of hemorrhage

and necrosis variably rimmed by schizont-infected macrophages were observed.[54,108] Multinucleated giant cells were rarely and inconsistently observed in the red pulp and within necrotic trabecular and white pulp arterioles.[123,125] Descriptions of lymph node histopathology are relatively scarce in wild artiodactyls with pathogenic theileriosis.[10,54,174] Variable numbers of pleomorphic, mono-, and/or multinucleated target cells were associated with thrombi and variable degrees of necrosis (Fig. 20).[10,54,123,174] Edema and congestion were rare.[123]

In the kidney, intravascular target cells were observed, especially in larger arteries,[54,123,125] with foci of necrosis and hemorrhage in some cases.[75,123,125] Glomerular[54] and perivascular (interstitial) target cell infiltrates have also been seen (Fig. 18).[10,75,123,125,174]

Target cells were reported within and/or surrounding the myocardial[10,54,75,123,125,193,194] and skeletal muscle[75] vasculature. Foci of myofiber degeneration and necrosis with or without associated hemorrhages have been described in cardiac (Fig. 19) and/or skeletal muscle.[10,75,123,125] Plugging of the myocardial vasculature with target cells and associated vasculitis was a rare and inconsistent finding.[123,125]

The gastrointestinal histopathology in wild artiodactyls with pathogenic theileriosis has been well described in gray/common duiker, eland, and giraffe.[54,108,123,125] Intra- and perivascular target cell infiltrates have been reported predominantly in the superficial abomasal mucosa (Fig. 21), where they were variably associated with erosions and ulcers, hemorrhage, and edema.[54,108] Transmural infiltration of target cells has been rarely described in the abomasum.[54] Foci of necrosis and hemorrhage were also observed in the small intestinal mucosa.[54,108] Arteritis and thrombosis was rarely and inconsistently associated with intravascular multinucleated target cells.[123,125]

Adrenal glands were not often sampled in wild artiodactyls that died of pathogenic theileriosis.[54,173,193,194] Reported lesions included intra- and perivascular target cells in the cortex and medulla (Fig. 22),[54,173,193,194] diffuse congestion,[123] associated foci of necrosis,[54] and multifocal hemorrhages.[123] Adrenal lesions were reportedly severe enough to cause adrenal dysfunction in 2 eland.[54]

Clinical neurologic abnormalities were not often reported, and the brain was uncommonly sampled, so central nervous system lesions have been rarely documented in wild artiodactyls with pathogenic theileriosis.[54,123,125] Mono- and/or multinucleated target cells were described in dilated arteries and arterioles, particularly in the choroid plexus, meninges, and midbrain (Fig. 23).[54,123,125] Congestion,[54,123,125] fibrin thrombi,[54] as well as foci of malacia/infarction[123,125] and hemorrhage[54,123,125] have been reported. These lesions are similar to those described in the brain and spinal cord of cattle with cerebral theileriosis/turning sickness caused by *T. taurotragi* and, rarely, also by *T. annulata* and *T. parva*.[14,33,187] However, due to the small sample size in wild artiodactyls, meaningful conclusions can be drawn only if the central nervous system histopathology is investigated in a greater number of wild

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‡‡‡‡References 10, 21, 54, 57, 75, 108, 123, 125, 128, 193, 194

ungulates, especially eland with fatal pathogenic theileriosis due to *T. taurotragi* infection.

Additional reported findings that may or may not be specific to theileriosis include variable sublethal vacuolar injury of hepatocytes,[75,108,123,125,174] splenic and lymph node lymphoid depletion with no discernible lymphoid follicles,[54,75] and stunting and fusion of intestinal villi.[54]

Detailed descriptions of the histopathology associated with pathogenic theileriosis are required in larger numbers of different wild artiodactyl species. The most detailed histopathologic descriptions are dated, and, in most cases, there was no molecular confirmation of theilerial infection. In addition, the target cell phenotype has not yet been established in organ sections from diverse species. As a result, the pathogenesis of transformative theileriosis is largely unknown in African wild artiodactyls.

An important histologic differential diagnosis for pathogenic theileriosis in wild artiodactyls is alcelaphine herpesvirus 1– or ovine herpesvirus 2–associated malignant catarrhal fever.[94] This disease has been reported in free-ranging and captive African buffaloes in South Africa, as well as in a variety of other captive and semicaptive wild artiodactyls.[144] Wild artiodactyls that die due to malignant catarrhal fever exhibit widespread lymphoid hyperplasia, multisystemic necrotizing vasculitis, and variable lymphoplasmacytic and histiocytic perivasculitis, often associated with parenchymal necrosis or mucosal ulceration.[144] Commonly affected organs include the kidneys, lymph nodes, spleen, hepatic portal tracts, brain, heart, gastrointestinal tract, trachea, and lungs.[144] Histopathology of the lesions and PCR (including RLB assays) on fresh spleen or lymph node samples is required to differentiate these diseases.

Leukocyte Phenotype and Pathogenesis

The transforming theilerias in domestic ruminants induce changes in a range of pivotal immune cells, resulting in an impaired immune-inflammatory response to the parasites,[31,38,45] which is fundamental to the proliferation, dissemination, and survival of target leukocytes in the host.[45,90,94] Target leukocytes differ in their phenotypic expression among theilerial species in domestic ungulates.[5,37,49,90,94,96,168] Early research used the differences in phenotypic expression between *T. parva*– and *T. annulata*–transformed leukocytes in cell culture to explain differences in pathogenesis and immune response.[49,96]

In vitro and ex vivo studies show that *T. parva* infects and transforms T and B lymphocytes but preferentially transforms T lymphocytes expressing CD4 or CD8 and either αβ or γδ T-cell receptors.[5,37] In contrast, *T. annulata* and closely related *T. lestoquardi* preferentially transform monocyte-macrophages, B lymphocytes, dendritic cells, and, to a lesser extent, T lymphocytes in cell culture.[31,37,89,90,96,168,172]

Recent studies that have investigated the leukocyte tropism of pathogenic *Theileria* species in vivo in domestic cattle suggest that the in vitro findings may be slightly misleading. For instance, in bovine tropical theileriosis[19] and ECF,[45] the proliferative foci in tissues consist largely of T lymphocytes and macrophages. In bovine cerebral theileriosis/turning sickness in Tanzanian cattle, histologic lesions in the brain were found to resemble lymphomatoid granulomatosis in humans, a nodular to diffuse angiocentric and angiodestructive granulomatous disease in the lungs, characterized by lymphocytes, macrophages, and atypical multinucleated giant cells.[14]

By using specific cell markers such as CD163, it was possible to show in ECF-infected cattle that macrophages likely play a significant role in the downregulation of a functional T-cell response and that key clinical signs and lesions may in fact be the result of a macrophage activation syndrome.[45] This syndrome has been implicated in the pathogenesis of diverse neoplastic, autoimmune, and infectious diseases in humans and animals, including bovine trypanosomiasis.[45]

Thus, recent in vivo studies indicate that subpopulations of T lymphocytes and macrophages/dendritic cells likely play a pivotal role in the pathogenesis of schizont-"transforming" theilerial diseases in domestic ruminants. The relationship among these leukocyte populations requires further study. Therefore, characterization of target leukocyte phenotypes in tissues from African wild artiodactyls with pathogenic theileriosis would contribute to our understanding of pathogenesis.

Aspects of Control

Currently, the "infection and treatment" method of immunization is the only available method of vaccination against ECF in cattle in Africa.[141] Cattle are vaccinated with a mixture of tick-derived *T. parva* stabilates (referred to as the Muguga cocktail), and they are treated concurrently with long-acting oxytetracycline, which prevents clinical disease but allows immunity to develop.[29,117,141] However, since oxytetracycline treatment is not protective against higher sporozoite doses in cattle, each new batch of sporozoite vaccine needs careful titration to establish the effective dose.[115] In addition, cattle that fail to respond effectively to oxytetracycline treatment may succumb to clinical disease unless treated with theilericidal drugs, such as buparvaquone, a naphthoquinone compound that is the drug of choice in domestic livestock with pathogenic theileriosis.[89,90,94]

Recently, a crude "infection and treatment" method with a tick-derived *Theileria sp.* (sable) stabilate was developed and used successfully to artificially expose captive-bred roan calves to infection.[175] However, the development of clinical disease in the calves meant that therapeutic intervention was required, which is a significant constraint to the practical application of this method under field conditions, especially in wild artiodactyls.[12,174] Postinfection treatment requires immobilization of sick calves, which is costly, logistically onerous, and could negatively affect their recovery. In addition, buparvaquone, the most effective treatment for pathogenic theileriosis in roan calves,[175] is not commercially available in South Africa because it induces a carrier state in Corridor disease, which is a controlled disease.[93]

Alternative, less invasive methods of controlled exposure in susceptible wild artiodactyls that do not require therapeutic intervention are needed.[12] One such method, which requires further investigation, is the inoculation of antelope calves with schizont-infected in vitro–cultured leukoblasts. Research shows that attenuation of *T. annulata* and *T. parva* occurs after multiple schizont-infected leukocyte replication cycles in cell culture.[115] In addition, inoculation of live attenuated *T. annulata* schizont-infected cell cultures confers protective immunity in cattle without causing clinical disease and therefore bypasses the requirement for treatment.[148] However, for reasons that are unclear, transfer of *T. parva* to cattle via inoculation of in vitro live attenuated cell cultures is not as effective.[18] A *Theileria sp.* (sable) schizont-infected leukocyte cell line exists with successful propagation of the parasite in vitro.[204] Preliminary studies on very few animals show that theilerial infection can be safely transmitted to roan calves via inoculation of an infected cell line and that calves appear to develop a degree of protective immunity.[12] However, this technique requires significant refinement and testing on a greater number of animals. Limiting factors include the scarcity and value of roan and sable antelope in particular and the possibility of incurring severe, potentially fatal disease.

Conclusion

It is likely that a variety of *Theileria* species infect the diverse wild ungulate species on the African continent. While several *Theileria* species have been identified in African wild artiodactyl species by phylogenetic analysis of the parasite 18S rRNA genes, many more probably remain to be discovered. Known *Theileria* species are commonly classified as pathogenic (schizont "transforming") or benign to mildly pathogenic ("nontransforming"). Normally a dynamic balance exists among wild mammalian hosts, tick vectors, *Theileria* parasites, and their environment, and as a result, the majority of animals in a population develop only subclinical infection. However, any perturbation of this balance can increase the susceptibility of wild ungulates to clinical disease and death. Similar to ECF in cattle, pathogenic theileriosis in wild artiodactyls is characterized by parasitic schizont-induced pseudo-neoplastic transformation, hyperproliferation, and dissemination of host leukocytes to multiple organs and the bloodstream. Histologically, leukoproliferative foci occur intra- and perivascularly in multiple organs and are associated with fibrin thrombi, necrosis, hemorrhage, and edema. More research is required to clarify the epidemiology and pathogenesis of theileriosis in African wild artiodactyls to prevent fatalities in susceptible species, particularly roan and sable antelope.

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References

1. Adamson R, Hall R. Virulence and attenuation in *Theileria annulata*. In: Dobbelaere DAE, McKeever DJ, eds. *Theileria*. Boston, MA: Kluwer Academic Publishers; 2002:55–67.

2. Allsopp BA, Baylis HA, Allsopp MT, et al. Discrimination between six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal RNA sequences. *Parasitology*. 1993;**107**(2):157–165.

3. Allsopp MTEP, Cavalier-Smith T, De Waal DT, et al. Phylogeny and evolution of the piroplasms. *Parasitology*. 1994;**108**(2):147–152.

4. Augustyn NJ, Bigalke RD. *Anaplasma* infection in a giraffe. *J S Afr Vet Assoc*. 1974;**45**(3):229.

5. Baldwin CL, Black SJ, Brown WC, et al. Bovine T cells, B cells, and null cells are transformed by the protozoan parasite *Theileria parva. Infect Immun*. 1988;**56**(2):462–467.

6. Barnett SF. Connective tissue reactions in acute fatal East Coast fever (*Theileria parva*) of cattle. *J Infect Dis*. 1960;**107**(3):253–282.

7. Barnett SF. *Theileria*. In: Kreier JP, ed. *Parasitic Protozoa*. Vol **4**. New York, NY: Academic Press; 1977:77–113.

8. Barnett SF, Brocklesby DW. Some piroplasms of wild mammals. *Symp Zool Soc London*. 1968;**24**:159–176.

9. Barnett SF, Brocklesby DW. The susceptibility of the African buffalo (*Syncerus caffer*) to infection with *Theileria parva* (Theiler, 1904). *Br Vet J*. 1966;**122**(9):379–386.

10. Basson PA, McCully RM, Kruger SP, et al. Parasitic and other diseases of the African buffalo in the Kruger National Park. *Onderstepoort J Vet Res*. 1970;**37**(1):11–28.

11. Beaumont E. East Coast fever of cattle. *Vet Rec*. 1939;**51**(44):1311–1312.

12. Benade JA. Immunization of roan antelope (*Hippotragus equinus*) using in vitro cultured *Theileria* species (sable) schizonts. MMedVet thesis. Pretoria, South Africa: University of Pretoria; 2010.

13. Berggoetz M, Schmid M, Ston D, et al. Tick-borne pathogens in the blood of wild and domestic ungulates in South Africa: interplay of game and livestock. *Ticks Tick Borne Dis*. 2014;**5**(2):166–175.

14. Biasibetti E, Sferra C, Lynen G, et al. Severe meningeal fibrinoid vasculitis associated with *Theileria taurotragi* infection in two short-horned zebu cattle. *Trop Anim Health Prod*. 2016;**48**(6):1297–1299.

15. Bigalke RD, Keep ME, Keep PJ, et al. A large *Babesia* sp. and a *Theileria*-like piroplasm of the square-lipped rhinoceros. *J S Afr Vet Med Assoc*. 1970;**41**(4):292–294.

16. Bigalke RD, Keep ME, Schoeman JH. Some protozoan parasites of tragelaphine antelopes in South Africa with special reference to a *Babesia* sp. in a bushbuck and a *Trypanosoma theileri*–like parasite in a nyala. *Onderstepoort J Vet Res*. 1972;**39**(4):225–228.

17. Bishop R, Musoke A, Morzaria S, et al. *Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitology*. 2004;**129**(suppl 7):271–283.

18. Boulter N, Hall R. Immunity and vaccine development in the bovine theilerioses. *Adv Parasitol*. 1999;**44**:41–97.

19. Branco S, Orvalho J, Leitão A, et al. Fatal cases of *Theileria annulata* infection in calves in Portugal associated with neoplastic-like lymphoid cell proliferation. *J Vet Sci*. 2010;**11**(1):27–34.

20. Brocklesby DW. A *Babesia* species of the black rhinoceros. *Vet Rec*. 1967;**80**(15):484.

21. Brocklesby DW. *Cytauxzoon taurotragi* Martin and Brocklesby, 1960, a piroplasm of eland (*Taurotragus oryx pattersonianus* Lydekker, 1906). *Res Vet Sci*. 1962;**3**(3):334–344.

22. Brocklesby DW. A new theilerial parasite of the African buffalo (*Syncerus caffer*). *Bull Epizoot Dis Africa*. 1965;**13**(4):325–330.

23. Brocklesby DW. Recent observations on tick-borne protozoa. In: Wilde JKH, ed. *Tick-Borne Diseases and Their Vectors*. Edinburgh, Scotland: Centre for Tropical Veterinary Medicine; 1978:263–286.

24. Brocklesby DW. Theileridae of the buffalo in East Africa. In: Corradetti A, ed. *Proceedings of the First International Congress of Parasitology, Roma, 21–26 September 1964*. Amsterdam, Netherlands: Pergamon (Elsevier Ltd); 1966:274–275.

25. Brocklesby DW, Barnett SF. The isolation of "*Theileria lawrencei* (Kenya)" from a wild buffalo (*Syncerus caffer*) and its serial passage through captive buffaloes. *Br Vet J*. 1966;**122**(9):387–395.

26. Brocklesby DW, Vidler BO. Haematozoa found in wild members of the order Artiodactyla in East Africa. *Bull Epizoot Dis Africa*. 1966;**14**(3):285–299.

27. Brocklesby DW, Vidler BO. Some parasites of East African wild animals. *Afr J Ecol*. 1965;**3**(1):120–122.

28. Brothers PS, Collins NE, Oosthuizen MC, et al. Occurrence of blood-borne tick-transmitted parasites in common tsessebe (*Damaliscus lunatus*) antelope in Northern Cape Province, South Africa. *Vet Parasitol*. 2011;**183**(1–2):160–165.

29. Burridge MJ, Morzaria SP, Cunningham MP, et al. Duration of immunity to East Coast fever (*Theileria parva* infection of cattle). *Parasitology*. 1972;**64**(3):511–515.

30. Burroughs REJ, Penzhorn BL, Wiesel I, et al. Piroplasms in brown hyaenas (*Parahyaena brunnea*) and spotted hyaenas (*Crocuta crocuta*) in Namibia and South Africa are closely related to *Babesia lengau*. *Parasitol Res*. 2017;**116**(2):685–692.

31. Campbell JDM, Spooner RL. Macrophages behaving badly: infected cells and subversion of immune responses to *Theileria annulata*. *Parasitol Today*. 1999;**15**(1):10–16.

32. Carmichael IH, Hobday E. Blood parasites of some wild bovidae in Botswana. *Onderstepoort J Vet Res*. 1975;**42**(2):55–62.

33. Catalano D, Biasibetti E, Lynen G, et al. "Ormilo disease" a disorder of zebu cattle in Tanzania: bovine cerebral theileriosis or new protozoan disease? *Trop Anim Health Prod*. 2015;**47**(5):895–901.

34. Chaisi ME, Janssens ME, Vermeiren L, et al. Evaluation of a real-time PCR test for the detection and discrimination of *Theileria* species in the African buffalo (*Syncerus caffer*). *Plos One*. 2013;**8**(10):e75827.

35. Cowdry EV, Danks WBC. Studies on East Coast fever: II. Behaviour of the parasite and the development of distinctive lesions in susceptible animals. *Parasitology*. 1933;**25**(1):1–63.

36. Dinnik JA, Walker JB, Barnett SF, et al. Some parasites obtained from game animals in western Uganda. *Bull Epizoot Dis Africa*. 1963;**11**(1):37–44.

37. Dobbelaere D, Baumgartner M. Theileria. In: Schaible UE, Haas A, eds. *Intracellular Niches of Microbes: A Pathogens Guide Through the Host Cell*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2009:613–632.

38. Dobbelaere D, Heussler V. Transformation of leukocytes by *Theileria parva* and *T annulata*. *Annu Rev Microbiol*. 1999;**53**:1–42.

39. Drevemo S, Grootenhuis JG, Karstad L. Blood parameters in wild ruminants in Kenya. *J Wildl Dis*. 1974;**10**(4):327–334.

40. Earnshaw A, Emerton L. The economics of wildlife tourism: theory and reality for landholders in Africa. In: Prins HHT, Grootenhuis JG, Dolan TT, eds. *Wildlife Conservation by Sustainable Use*. Dordrecht, Netherlands: Springer; 2000:315–334.

41. Espinaze MPA, Hellard E, Horak IG, et al. Domestic mammals facilitate tick-borne pathogen transmission networks in South African wildlife. *Biol Conserv*. 2018;**221**:228–236.

42. Fawcett DW. Life cycle of *Theileria parva*, the parasitic protozoan that causes East Coast fever in cattle. The tick species *R. appendiculatus* is the main vector for the disease. London, England: Science Photo Library. C022/0963

43. Fawcett DW, Conrad PA, Grootenhuis JG, et al. Ultrastructure of the intra-erythrocytic stage of *Theileria* species from cattle and waterbuck. *Tissue Cell*. 1987;**19**(5):643–655.

44. Fivaz BH. Immune suppression induced by the brown ear tick *Rhipicephalus appendiculatus* Neumann, 1901. *J Parasitol*. 1989;**75**(6):946–952.

45. Fry LM, Schneider DA, Frevert CW, et al. East Coast Fever caused by *Theileria parva* is characterized by macrophage activation associated with vasculitis and respiratory failure. *PLoS One*. 2016;**11**(5):e0156004.

46. Gachohi J, Skilton R, Hansen F, et al. Epidemiology of East Coast fever (*Theileria parva* infection) in Kenya: past, present and the future. *Parasit Vectors*. 2012;**5**:194.

47. Githaka N, Konnai S, Bishop R, et al. Identification and sequence characterization of novel *Theileria* genotypes from the waterbuck (*Kobus defassa*) in a *Theileria parva*–endemic area in Kenya. *Vet Parasitol*. 2014;**202**(3–4):180–193.

48. Glass EJ. The balance between protective immunity and pathogenesis in tropical theileriosis: what we need to know to design effective vaccines for the future. *Res Vet Sci*. 2001;**70**(1):71–75.

49. Glass EJ, Innes EA, Spooner RL, et al. Infection of bovine monocyte/macrophage populations with *Theileria annulata* and *Theileria parva*. *Vet Immunol Immunopathol*. 1989;**22**(4):355–368.

50. Goh S, Ngugi D, Lizundia R, et al. Identification of *Theileria lestoquardi* antigens recognized by CD8+ T cells. *Plos One*. 2016;**11**:e0162571.

51. Gray CE, Robertson W. Texas fever or redwater in Rhodesia. *J Comp Pathol Ther*. 1902;**15**(4):368–378.

52. Grobler JH. Parasites and mortality of sable *Hippotragus niger niger* (Harris, 1838) in the Matopos, Zimbabwe. *Koedoe*. 1981;**24**(1):119–123.

53. Grootenhuis JG, Leitch BL, Stagg DA, et al. Experimental induction of *Theileria parva lawrencei* carrier state in an African buffalo (*Syncerus caffer*). *Parasitology*. 1987;**94**(3):425–431.

54. Grootenhuis JG, Morrison WI, Karstad L, et al. Fatal theileriosis in eland (*Taurotragus oryx*): pathology of natural and experimental cases. *Res Vet Sci*. 1980;**29**(2):219–229.

55. Grootenhuis JG, Young AS. The importance of wildlife in the epidemiology of theileriosis. In: Karstad L, Nestel B, Graham H, eds. *Wildlife Disease Research and Economic Development*. Ottawa, Canada: International Development Research Centre; 1981:33–39.

56. Grootenhuis JG, Young AS. The involvement of wildlife in *Theileria* infections of domestic animals in East Africa. In: Irvin AD, Cunningham MP, Young AS, eds. *Advances in the Control of Theileriosis*. The Hague, Netherlands: Martinus Nijhoff; 1981:71–73.

57. Grootenhuis JG, Young AS, Dolan TT, et al. Characteristics of *Theileria* species (eland) infections in eland and cattle. *Res Vet Sci*. 1979;**27**(1):59–68.

58. Grootenhuis JG, Young AS, Kanhai GK, et al. Experimental tick transmission of *Theileria* species between eland. *J Parasitol*. 1977;**63**(6):1127–1129.

59. Grootenhuis JG, Young AS, Kimber CD, et al. Investigations on a *Theileria* species from an impala. *J Wildl Dis*. 1975;**11**(1):122–127.

60. Grootenhuis JG, Young AS, Stagg DA, et al. Infection of African buffalo (*Syncerus caffer*) and cattle with *Theileria parva* lawrencei after serial passage in cattle. *Res Vet Sci*. 1987;**42**(3):326–330.

61. Grootenhuis JG, Young AS, Uilenberg G. The relationship between *Theileria taurotragi* from eland and *Theileria* sp. (Idobogo) from cattle. *Vet Parasitol*. 1981;**8**(1):39–47.

62. Gubbels JM, de Vos AP, van der Weide M, et al. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J Clin Microbiol*. 1999;**37**(6):1782–1789.

63. Harris DJ, Halajian A, Santos JL, et al. Diversity of haemoprotozoan parasites infecting the wildlife of South Africa. *Folia Parasitol*. 2018;**65**:2018.015.

64. Hay SI. The paradox of endemic stability. *Trends Parasitol*. 2001;**17**(7):310.

65. Heussler VT, Stanway RR. Cellular and molecular interactions between the apicomplexan parasites *Plasmodium* and *Theileria* and their host cells. *Parasite*. 2008;**15**(3):211–218.

66. Hill RR, Condy JB, Matson BA. *Theileria*-type piroplasms of Rhodesian Artiodactyla. *Vet Rec*. 1969;**84**(11):287–288.

67. Hing S, Narayan EJ, Thompson RCA, et al. The relationship between physiological stress and wildlife disease: consequences for health and conservation. *Wildl Res*. 2016;**43**(1):51–60.

68. Horak IG, Anthonissen M, Krecek RC, et al. Arthropod parasites of springbok, gemsbok, kudus, giraffes and Burchell's and Hartmann's zebras in the Etosha and Hardap Nature Reserves, Namibia. *Onderstepoort J Vet Res*. 1992;**59**(4): 253–257.

69. Hove T, Sithole N, Munodzana D, et al. Isolation and characterization of a *Babesia* species from *Rhipicephalus evertsi evertsi* ticks picked off a sable antelope (*Hippotragus niger*) which died of acute babesiosis. *Onderstepoort J Vet Res*. 1998;**65**(2):75–80.

70. El Imam AH. Pathogenesis and susceptibility of sheep to *Theileria lestoquardi* and molecular detection of other ovine *Theileria* species in the Sudan. PhD thesis. Khartoum, Sudan: University of Khartoum; 2010.

71. El Imam AH, Taha KM. Malignant ovine theileriosis (*Theileria lestoquardi*): a review. *Jordan J Biol Sci*. 2015;**8**(3):165–174.

72. Irvin AD, Mwamachi DM. Clinical and diagnostic features of East Coast fever (*Theileria parva*) infection of cattle. *Vet Rec*. 1983;**113**(9):192–198.

73. Irvin AD, Omwoyo P, Purnell RE, et al. Blood parasites of impala (*Aepyceros melampus*) in the Serengeti National Park. *Vet Rec*. 1973;**93**(7):200–203.

74. Irvin AD, Peirce MA, Purnell RE, et al. The possible role of the eland (*Taurotragus oryx*) in the epidemiology of East Coast fever and other bovine theilerioses. *Vet Rec*. 1972;**91**(21):513–517.

75. Jardine JE. The pathology of cytauxzoonosis in a tsessebe (*Damaliscus lunatus*). *J S Afr Vet Assoc*. 1992;**63**(1):49–51.

76. Jonsson NN, Bock RE, Jorgensen WK, et al. Is endemic stability of tick-borne disease in cattle a useful concept? *Trends Parasitol*. 2012;**28**(3):85–89.

77. Kaleibar MT, Helan JA, Fathi E. Occurrence of congenital cerebral theileriosis in a newborn twin Holstein calves in Iran: case report. *Vet Res Forum*. 2014; **5**(3):237–241.

78. Karbe E, Grootenhuis JG, Kelley S, et al. Experiments on the *Babesia bigemina* carrier state in East African buffalo and eland. *Tropenmed Parasitol*. 1979; **30**(3):313–317.

79. Kazimírová M, Štibrániová I. Tick salivary compounds: their role in modulation of host defences and pathogen transmission. *Front Cell Infect Microbiol*. 2013; **3**:43.

80. Kivaria FM. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. *Trop Anim Health Prod*. 2006;**38**(4):291–299.

81. Koch R. Rhodesian redwater, or African Coast fever. *Vet J*. 1904;**58**(5): 251–260.

82. de Kock G. Studies on the lesions and pathogenesis of East Coast fever (*Theileria parva* infection) in cattle, with special reference to the lymphoid tissue. *Onderstepoort J Vet Res*. 1957;**27**(3):431–452.

83. Krecek RC, Boomker J, Penzhorn BL, Scheepers L. Internal parasites of giraffes (*Giraffa camelopardalis angolensis*) from Etosha National Park, Namibia. *J Wildl Dis*. 1990;**26**(3):395–397.

84. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;**33**(7):1870–1874.

85. Kuttler KL. *Anaplasma* infections in wild and domestic ruminants: a review. *J Wildl Dis*. 1984;**20**(1):12–20.

86. Latif AA, Hove T, Kanhai GK, et al. Buffalo-associated *Theileria parva*: the risk to cattle of buffalo translocation into the highveld of Zimbabwe. *Ann N Y Acad Sci*. 2002;**969**:275–279.

87. Lawrence JA, Byaruhanga C, Oosthuizen M, et al. Non-pathogenic *Theileria* species in cattle. In: Coetzer JAW, Thomson GR, Maclachlan J, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

88. Lawrence JA, Byaruhanga C, Oosthuizen M, et al. *Theileria mutans* infection. In: Coetzer JAW, Thompson GR, Maclachlan NJ, eds. *Infectious diseases of livestock*. Pretoria, South Africa: Anipedia; 2017.

89. Lawrence JA, Byaruhanga C, Oosthuizen M, et al. Theileriosis of sheep and goats. In: Coetzer JAW, Thomson GR, Maclachlan J, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

90. Lawrence JA, Mans BJ. *Theileria annulata* theileriosis. In: Coetzer JAW, Thomson GR, Maclachlan J, Penrith ML, Michel A, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

91. Lawrence JA, Mans BJ. Theileria buffeli/orientalis infection. In: Coetzer JAW, Thomson GR, Maclachlan NJ, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

92. Lawrence JA, Mans BJ. Turning sickness. In: Coetzer JAW, Thomson GR, Maclachlan J, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

93. Lawrence JA, Sibeko-Matjila KP, Mans BJ. Corridor disease. In: Coetzer JAW, Thomson GR, Maclachlan J, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

94. Lawrence JA, Sibeko-Matjila KP, Mans BJ. East Coast fever. In: Coetzer JAW, Thomson GR, Maclachlan J, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

95. Lawrence JA, Sibeko-Matjila KP, Mans BJ. Zimbabwe theileriosis. In: Coetzer JAW, Thomson GR, Maclachlan NJ, eds. *Infectious Diseases of Livestock*. Pretoria, South Africa: Anipedia; 2017.

96. Leemans I, Fossum C, Johannisson A, et al. Comparative studies on surface phenotypes of *Theileria lestoquardi* and *T. annulata* schizont-infected cells. *Parasitol Res*. 2001;**87**(9):768–777.

97. Levine ND. Taxonomy of the piroplasms. *Trans Am Microsc Soc*. 1971;**90**(1): 2–33.

98. Lewis EA. East Coast fever and the African buffalo, the eland and the bushbuck. *East African Agric J*. 1943;**9**(2):90–92.

99. Lichtenheld G. Beurteilung eines befundes von Kochsen plasmakugeln in niereninfarkten einer elenantilope. *Zeitschrift für Infekt Parasitäre Krankheiten und Hyg der Haustiere*. 1911;**9**:155–156.

100. Löhr KF, Meyer H. Game anaplasmosis: the isolation of *Anaplasma* organisms from antelope. *Z Tropenmed Parasitol*. 1973;**24**(2):192–197.

101. Makala LH, Mangani P, Fujisaki K, et al. The current status of major tick borne diseases in Zambia. *Vet Res*. 2003;**34**(1):27–45.

102. Mans BJ, Pienaar R, Latif AA. A review of *Theileria* diagnostics and epidemiology. *Int J Parasitol Parasites Wildl*. 2015;**4**(1):104–118.

103. Mans BJ, Pienaar R, Latif AA, et al. Diversity in the 18 S SSU rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. *Parasitology*. 2011;**138**(6):766–779.

104. Martin H, Brocklesby DW. A new parasite of eland. *Vet Rec*. 1960;**72**(17): 331–332.

105. Martin LB, Andreassi E, Watson W, et al. Stress and animal health: physiological mechanisms and ecological consequences. *Nat Educ Knowl*. 2011;**3**(6): 11.

106. Martinaglia G. Red-water (babesiosis) in a sable antelope. *J S Afr Vet Med Assoc*. 1930;**1**(4):41–42.

107. Matjila PT, Leisewitz AL, Oosthuizen MC, et al. Detection of a *Theileria* species in dogs in South Africa. *Vet Parasitol*. 2008;**157**(1–2):34–40.

108. McCully RM, Keep ME, Basson PA. Cytauxzoonosis in a giraffe [*Giraffa camelopardalis* (Linnaeus, 1758)] in Zululand. *Onderstepoort J Vet Res*. 1970;**37**(1):7–10.

109. McInnes EF, Stewart CG, Penzhorn BL, et al. An outbreak of babesiosis in imported sable antelope (*Hippotragus niger*). *J S Afr Vet Assoc*. 1991;**62**(1): 30–32.

110. McKeever DJ. Bovine immunity—a driver for diversity in *Theileria* parasites? *Trends Parasitol*. 2009;**25**(6):269–276.

111. McLeod A, Kristjanson R. *Impact of Ticks and Associated Diseases on Cattle in Asia, Australia and Africa. ILRI and eSYS Report to ACIAR*. Nairobi, Kenya: International Livestock Research Institute; 1999.

112. Melhorn H, Schein E. The piroplasms: life cycle and sexual stages. *Adv Parasitol*. 1984;**23**:37–103.

113. Melton DA, Melton CL. Condition and mortality of waterbuck (*Kobus ellipsiprymnus*) in the Umfolozi Game Reserve. *Afr J Ecol*. 1982;**20**(2):89–103.

114. Mohan RN, Gotts MG. Diseases and parasites of the African buffalo (*Syncerus caffer*). *Vet Bull*. 1970;**40**(3):157–165.

115. Morrison WI, McKeever DJ. Current status of vaccine development against *Theileria* parasites. *Parasitology*. 2006;**133**(suppl 2):169–187.

116. Morrison WI, Taracha ELN, McKeever DJ. Contribution of T-cell responses to immunity and pathogenesis in infections with *Theileria parva*. *Parasitol Today*. 1995;**11**(1):14–18.

117. Morzaria SP, Irvin AD, Voigt WP, et al. Effect of timing and intensity of challenge following immunization against East Coast fever. *Vet Parasitol*. 1987;**26**(1–2):29–41.

118. Neitz WO. Blood parasites of game in Zululand. *17th Report of the Director of Veterinary Services and Animal Industry*. Union of South Africa; 1931.

119. Neitz WO. Blood parasites of game in Zululand: further report. *Onderstepoort J Vet Sci Anim Ind*. 1933;**1**(2):411–417.

120. Neitz WO. Bovine anaplasmosis: the transmission of *Anaplasma marginale* to a black wildebeest (*Conochaetes gnu*). *Onderstepoort J Vet Res*. 1935;**5**(1):9–11.

121. Neitz WO. *The Different Forms of Tick Toxicosis: A review. Second Meeting of the FAO/OIE Expert Panel of Tick-Borne Diseases of Livestock*. Cairo, Egypt; 1963.

122. Neitz WO. The epidemiological pattern of viral, protophytal and protozoal zoonoses in relation to game preservation in South Africa. *J S Afr Vet Med Assoc*. 1967;**38**(2):129–141.

123. Neitz WO. Theileriosis, gonderioses and cytauxzoonoses: a review. *Onderstepoort J Vet Res*. 1957;**27**(3):275–430.

124. Neitz WO, Canham AS, Kluge EB. Corridor disease: a fatal form of bovine theileriosis encountered in Zululand. *J S Afr Vet Med Assoc*. 1955;**26**(2):79–88.

125. Neitz WO, Thomas AD. *Cytauxzoon sylvicaprae* gen nov, spec nov, a protozoon responsible for a hitherto undescribed disease in the duiker [*Sylvicapra grimmia* (Linne)]. *Onderstepoort J Vet Sci*. 1948;**23**(1–2):63–76.

126. Nene V, Kiara H, Lacasta A, et al. The biology of *Theileria parva* and control of East Coast fever—current status and future trends. *Ticks Tick Borne Dis*. 2016;**7**(4):549–564.

127. Nijhof AM, Penzhorn BL, Lynen G, et al. *Babesia bicornis* sp nov and *Theileria bicornis* sp nov: tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). *J Clin Microbiol*. 2003;**41**(5):2249–2254.

128. Nijhof AM, Pillay V, Steyl J, et al. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. *J Clin Microbiol*. 2005;**43**(12):5907–5911.

129. Norval RAI, Perry BD, Young AS. *The Epidemiology of Theileriosis in Africa*. London, England: Academic Press; 1992.

130. Oberem PT, Bezuidenhout JD. Heartwater in hosts other than domestic ruminants. *Onderstepoort J Vet Res*. 1987;**54**(3):271–275.

131. Odongo DO, Ueti MW, Mwaura SN, et al. Quantification of *Theileria parva* in *Rhipicephalus appendiculatus* (Acari: Ixodidae) confirms differences in infection between selected tick strains. *J Med Entomol*. 2009;**46**(4):888–894.

132. O'Donoghue P. Haemoprotozoa: making biological sense of molecular phylogenies. *Int J Parasitol Parasites Wildl*. 2017;**6**(3):241–256.

133. Okello MM. Economic contribution, challenges and way forward for wildlife-based tourism industry in eastern African countries. *J Tour Hosp*. 2014;**3**(1).122.

134. Olwoch JM, Reyers B, Van Jaarsveld AS. Host-parasite distribution patterns under simulated climate: implications for tick-borne diseases. *Int J Climatol*. 2009;**29**(suppl 7):993–1000.

135. Olwoch JM, Van Jaarsveld AS, Scholtz CH, et al. Climate change and the genus *Rhipicephalus* (Acari: Ixodidae) in Africa. *Onderstepoort J Vet Res*. 2007;**74**(1):45–72.

136. Oosthuizen MC, Allsopp BA, Troskie M, et al. Identification of novel *Babesia* and *Theileria* species in South African giraffe (*Giraffa camelopardalis*, Linnaeus, 1758) and roan antelope (*Hippotragus equinus*, Desmarest 1804). *Vet Parasitol*. 2009;**163**(1–2):39–46.

137. Oosthuizen MC, Zweygarth E, Collins NE, et al. Identification of a novel *Babesia* sp from a sable antelope (*Hippotragus niger* Harris, 1838). *J Clin Microbiol*. 2008;**46**(7):2247–2251.

138. Oura CA, Tait A, Asiimwe B, et al. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. *Parasitol Res*. 2011;**108**(6):1365–1374.

139. Parsons SDC, Penzhorn BL, Reyers F, et al. Erythrocyte morphology and haemoglobin types of neonatal roan antelopes (*Hippotragus equinus*) with hypochromic poikilocytic anaemia. *J Comp Pathol*. 2006;**134**(2–3):152–160.

140. Pascucci I, Capobianco Dondona A, Camma C, et al. Survey of ixodid ticks and two tick-borne pathogens in African buffaloes, *Syncerus caffer*, from the Caprivi Strip, Namibia. *J Zoo Wildl Med*. 2011;**42**(4):634–640.

141. Patel E, Mwaura S, Di Giulio G, et al. Infection and treatment method (ITM) vaccine against East Coast fever: reducing the number of doses per straw for use in smallholder dairy herds by thawing, diluting and refreezing already packaged vaccine. *BMC Vet Res*. 2019;**15**(1):46.

142. Penzhorn BL. Babesiosis of wild carnivores and ungulates. *Vet Parasitol*. 2006;**138**(1–2):11–21.

143. Peter TF, Burridge MJ, Mahan SM. *Ehrlichia ruminantium* infection (heartwater) in wild animals. *Trends Parasitol*. 2002;**18**(5):214–218.

144. Pfitzer S, Last R, Espie I, et al. Malignant catarrhal fever: an emerging disease in the African buffalo (*Syncerus caffer*). *Transbound Emerg Dis*. 2015;**62**(3):288–294.

145. Pfitzer S, Oosthuizen MC, Bosman AM, et al. Tick-borne blood parasites in nyala (*Tragelaphus angasii*, Gray 1849) from KwaZulu-Natal, South Africa. *Vet Parasitol*. 2011;**176**(2–3):126–131.

146. Pienaar R, Latif AA, Thekisoe OM, et al. Geographic distribution of *Theileria* sp (buffalo) and *Theileria* sp (bougasvlei) in Cape buffalo (*Syncerus caffer*) in southern Africa: implications for speciation. *Parasitology*. 2014;**141**(3):411–424.

147. Pienaar R, Potgieter FT, Latif AA, et al. Mixed *Theileria* infections in free-ranging buffalo herds: implications for diagnosing *Theileria parva* infections in Cape buffalo (*Syncerus caffer*). *Parasitology*. 2011;**138**(7):884–895.

148. Pipano E, Samish M, Kriegel Y, Yeruham I. Immunization of Friesian cattle against *Theileria annulata* by the infection-treatment method. *Br Vet J*. 1981;**137**(4):416–420.

149. Pospisil J, Kase F, Vahala J, et al. Basic hematological values in antelopes. 1. The Aepycerotinae and the Alcelaphinae. *Comp Biochem Physiol A Physiology*. 1984;**78**(3):547–551.

150. Pospisil J, Kase F, Vahala J, et al. Basic hematological values in antelopes. 2. The Hippotraginae and the Tragelaphinae. *Comp Biochem Physiol A Physiology*. 1984;**78**(4):799–807.

151. Pospisil J, Kase F, Vahala J, et al. Basic hematological values in antelopes. 3. The Reduncinae and the Antelopinae. *Comp Biochem Physiol A Physiology*. 1984;**78**(4):809–813.

152. Pospisil J, Kase F, Vahala J, et al. Basic hematological values in antelopes. 4. A comparison and concluding remarks. *Comp Biochem Physiol A Physiology*. 1984;**78**(4):815–821.

153. Potgieter FT, Stoltsz WH, Blouin EF, et al. Corridor disease in South Africa: a review of the current status. *J South African Vet Assoc*. 1988;**59**(3):155–160.

154. Preston PM, Hall FR, Glass EJ, et al. Innate and adaptive immune responses co-operate to protect cattle against *Theileria annulata*. *Parasitol Today*. 1999;**15**(7):268–274.

155. van Rensburg IBJ. Bovine cerebral theileriosis: a report on five cases with splenic infarction. *J S Afr Vet Assoc*. 1976;**47**(2):137–141.

156. van Rensburg SJ. Haematological investigations into the rhipicephaline tick toxicosis syndrome. *J S Afr Vet Med Assoc*. 1959;**30**(2):75–95.

157. Ros-García A, Barandika JF, García-Pérez AL, et al. Assessment of exposure to piroplasms in sheep grazing in communal mountain pastures by using a multiplex DNA bead-based suspension array. *Parasit Vectors*. 2013;**6**(1):277.

158. Ros-García A, Juste RA, Hurtado A. A highly sensitive DNA bead-based suspension array for the detection and species identification of bovine piroplasms. *Int J Parasitol*. 2012;**42**(2):207–214.

159. Ross PH. East Coast fever. Vol **1**. *Nairobi Laboratory Reports for 1904 to 1910*. Nairobi, Kenya; 1911.

160. Schnittger L, Yin H, Qi B, et al. Simultaneous detection and differentiation of *Theileria* and *Babesia* parasites infecting small ruminants by reverse line blotting. *Parasitol Res*. 2004;**92**(3):189–196.

161. Schreeg ME, Marr HS, Tarigo JL, et al. Mitochondrial genome sequences and structures aid in the resolution of Piroplasmida phylogeny. *Plos One*. 2016;**11**(11): e0165702.

162. Shaw MK. Cell invasion by *Theileria* sporozoites. *Trends Parasitol*. 2003;**19**(1):2–6.

163. Shaw MK. *Theileria* development and host cell invasion. In: Dobbelaere DAE, McKeever DJ, eds. *Theileria*. Boston, MA: Kluwer Academic Publishers; 2002:1–22.

164. Sibeko KP, Oosthuizen MC, Collins NE, et al. Development and evaluation of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. *Vet Parasitol*. 2008;**155**(1–2):37–48.

165. Silberová P, Lukešová D, Pinc L. The determination of blood profile in captive wild ungulates with focus on eland (*Taurotragus oryx*). *Agric Trop Subtrop*. 2010;**43**(4):360–365.

166. Sivakumar T, Hayashida K, Sugimoto C, et al. Evolution and genetic diversity of *Theileria*. *Infect Genet Evol*. 2014;**27**:250–263.

167. Spitalska E, Riddell M, Heyne H, et al. Prevalence of theileriosis in Red Hartebeest (*Alcelaphus buselaphus caama*) in Namibia. *Parasitol Res*. 2005;**97**(1):77–79.

168. Spooner RL, Innes EA, Glass EJ, et al. *Theileria annulata* and *T. parva* infect and transform different bovine mononuclear cells. *Immunology*. 1989;**66**(2):284–288.

169. Stagg DA, Bishop RP, Morzaria SP, et al. Characterization of *Theileria parva* which infects waterbuck (*Kobus defassa*). *Parasitology*. 1994;**108**(5):543–554.

170. Stagg DA, Kanhai GK, Young AS, et al. The establishment of *Theileria*-infected cell lines from an eland (*Taurotragus oryx*, Lydekker 1906). *Res Vet Sci*. 1976;**20**(2):122–126.

171. Stagg DA, Young AS, Leitch BL, et al. Infection of mammalian cells with *Theileria* species. *Parasitology*. 1983;**86**(2):243–254.

172. Stephens SA, Howard CJ. Infection and transformation of dendritic cells from bovine afferent lymph by *Theileria annulata*. *Parasitology*. 2002;**124**(5):485–493.

173. Steyl J. Theileriosis in roan antelope. *Newsletter of the Wildlife Group of the South African Veterinary Association*. 2003;**7**:4–9.

174. Steyl JCA. Theileriosis in roan antelope (*Hippotragus equinus*): identification of vectors and experimental transmission using a tick-derived stabilate. MSc Thesis. Pretoria, South Africa: University of Pretoria; 2012.

175. Steyl JCA, Prozesky L, Stoltsz WH, et al. Theileriosis (cytauxzoonosis) in Roan antelope (*Hippotragus equinus*): field exposure to infection and identification of potential vectors. *Onderstepoort J Vet Res*. 2012;**79**(1):E1–E8.

176. Stoltsz WH, Dunsterville MT. In vitro establishment and cultivation of a *Cytauxzoon* sp (*Theileria* sp) from a sable antelope (*Hippotragus niger*, Harris 1838). *J S Afr Vet Assoc*. 1992;**63**(4):182.

177. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*. 1993;**10**(3):512–526.

178. Taracha EL, Awino E, McKeever DJ. Distinct CD4+ T cell helper requirements in *Theileria parva*–immune and –naive bovine CTL precursors. *J Immunol*. 1997;**159**(9):4539–4545.

179. Theiler A. *The Artificial Transmission of East Coast Fever: Report of the Government Veterinary Bacteriologist for the Year 1909–1910*. Pretoria, South Africa; 1911.

180. Thomas AD, Neitz WO. Rhipicephaline tick toxicosis in cattle: its possible aggravating effects on certain diseases. *J S Afr Vet Assoc*. 1958;**29**(1):39–50.

181. Thomas SE, Wilson DE, Mason TE. *Babesia, Theileria* and *Anaplasma spp*. infecting sable antelope, *Hippotragus niger* (Harris, 1838), in southern Africa. *Onderstepoort J Vet Res*. 1982;**49**(3):163–166.

182. Tizard IR. Immunity in the fetus and newborn. In: *Veterinary Immunology*. St Louis, MO: Elsevier; 2018:247–260.

183. Todd JL, Wolbach SB. Parasitic protozoa from the Gambia. *J Med Res*. 1912;**26**(2):195–218.

184. Tonetti N, Berggoetz M, Ruhle C, et al. Ticks and tick-borne pathogens from wildlife in the Free State Province, South Africa. *J Wildl Dis*. 2009;**45**(2):437–446.

185. Tretina K, Gotia HT, Mann DJ, et al. *Theileria*-transformed bovine leukocytes have cancer hallmarks. *Trends Parasitol*. 2015;**31**(7):306–314.

186. Turner WC, Küsters M, Versfeld W, et al. Ixodid tick diversity on wild mammals, birds, and reptiles in and around Etosha National Park, Namibia. *Afr J Ecol*. 2017;**55**(4):714–721.

187. Tustin RC, van Heerden J. Bovine cerebral theileriosis (turning sickness) with spinal cord involvement. *J S Afr Vet Assoc*. 1979;**50**(1):49–51.

188. Uilenberg G. *Theileria* species of domestic livestock. In: Irvin AD, Cunningham MP, Young AS, eds. *Advances in the Control of Theileriosis*. The Hague, Netherlands: Martinus Nijhoff; 1981:4–37.

189. Uys AC, Horak IG, Harrison A. Questing ixodid ticks on the vegetation of sable antelope and multi-herbivore enclosures in Thabazimbi. *J S Afr Vet Assoc*. 2015;**86**(1):1243.

190. Vudriko P, Okwee-Acai J, Tayebwa DS, et al. Emergence of multi-acaricide resistant *Rhipicephalus* ticks and its implication on chemical tick control in Uganda. *Parasit Vectors*. 2016;**9**:4.

191. de Waal DT. Anaplasmosis control and diagnosis in South Africa. *Ann N Y Acad Sci*. 2000;**916**(1):474–483.

192. Wamuyu L, Obanda V, Kariuki D, et al. Molecular detection and characterization of *Theileria* infecting wildebeest (*Connochaetes taurinus*) in the Masai Mara National Reserve, Kenya. *Pathogens*. 2015;**4**(3):626–638.

193. Wilson DE, Bartsch RC, Bigalke RD, Thomas SE. Observations on mortality rates and disease in roan and sable antelope on nature reserves in the Transvaal. *J South African Wildl Manag Assoc*. 1974;**4**(3):203–206.

194. Wilson DE, Hirst SM. Ecology and factors limiting roan and sable antelope populations in South Africa. *Wildl Monogr*. 1977;**54**(1):3–111.

195. Ye J, Coulouris G, Zaretskaya I, et al. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*. 2012;**13**:134.

196. Young AS. The epidemiology of theileriosis in East Africa. In: Irvin AD, Cunningham MP, Young AS, eds. *Advances in the Control of Theileriosis: Proceedings of an International Conference Held at the International Laboratory for Research on Animal Diseases in Nairobi, 9–13th February, 1981*. The Hague, Netherlands: Martinus Nijhoff Publishers; 1981:38–55.

197. Young AS, Brown CG, Burridge MJ, et al. The incidence of theilerial parasites in East African buffalo (*Syncerus caffer*). *Tropenmed Parasitol*. 1978;**29**(3):281–288.

198. Young AS, Grootenhuis JG. Influence of wildlife on immunization of cattle against theileriosis in East Africa. In: Irvin AD, ed. *Immunization Against Theileriosis Africa*. Nairobi, Kenya: International Laboratory for Research on Animal Diseases; 1985:104–109.

199. Young AS, Grootenhuis JG, Kimber CD, et al. Isolation of a *Theileria* species from eland (*Taurotragus oryx*) infective for cattle. *Tropenmed Parasitol*. 1977;**27**(2):185–194.

200. Young AS, Grootenhuis JG, Leitch BL, et al. The development of *Theileria = Cytauxzoon taurotragi* (Martin and Brocklesby, 1960) from eland in its tick vector *Rhipicephalus appendiculatus*. *Parasitology*. 1980;**81**(1):129–144.

201. Young AS, Grootenhuis JG, Smith K, et al. Structures associated with *Theileria* parasites in eland erythrocytes. *Ann Trop Med Parasitol*. 1978;**72**(5): 443–454.

202. Yusufmia SB, Collins NE, Nkuna R, et al. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. *J S Afr Vet Assoc*. 2010;**81**(1):45–49.

203. Zhang Y, Li T, Cui Y, et al. The first report of *Anaplasma phagocytophilum* and a novel *Theileria spp.* co-infection in a South African giraffe. *Parasitol Int*. 2016;**65**(4):347–351.

204. Zweygarth E, Benade J, Steyl J, et al. *In vitro* cultivation of a *Theileria* species from a roan antelope (*Hippotragus equinus*). *Parasitol Res*. 2009;**105**(6): 1755–1757.

205. Zweygarth E, Koekemoer O, Josemans AI, et al. *Theileria*-infected cell line from an African buffalo (*Syncerus caffer*). *Parasitol Res*. 2009;**105**(2): 579–581.