The Piroplasmida *Babesia*, *Cytauxzoon*, and *Theileria* in farm and companion animals: species compilation, molecular phylogeny, and evolutionary insights

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Abstract

The order Piroplasmida, including the genera *Babesia*, *Cytauxzoon*, and *Theileria* is often referred to as piroplasmids and comprises of dixenous hemoprotozoans transmitted by ticks to a mammalian or avian host. Although piroplasmid infections are usually asymptomatic in wild animals, in domestic animals, they cause serious or life-threatening consequences resulting in fatalities. Piroplasmids are particularly notorious for the enormous economic loss they cause worldwide in livestock production, the restrictions they pose on horse trade, and the negative health impact they have on dogs and cats. Furthermore, an increasing number of reported human babesiosis cases are of growing concern. Considerable international research and epidemiological studies are done to identify existing parasite species, reveal their phylogenetic relationships, and develop improved or new drugs and vaccines to mitigate their impact. In this review, we present a compilation of all piroplasmid species, isolates, and species complexes that infect domestic mammals and which have been well defined by molecular phylogenetic markers. Altogether, 57 taxonomic piroplasmid entities were compiled, comprising of 43 piroplasmid species, 12 well-defined isolates awaiting formal species description, and two species complexes that possibly mask additional species. The extrapolation of the finding of at least 57 piroplasmid species in only six domestic mammalian groups (cattle, sheep, goat, horse, dog, and cat) allows us to predict that a substantially higher number of piroplasmid parasites than vertebrate host species exist. Accordingly, the infection of a vertebrate host species by multiple piroplasmid species from the same and/or different phylogenetic lineages is commonly observed. Molecular phylogeny using 18S rRNA genes of piroplasmids infecting domestic mammals results in the formation of six clades, which emerge due to an anthropocentric research scope, but not due to a possibly assumed biological priority position. Scrutinizing the topology of inferred trees reveals stunning insights into some evolutionary patterns exhibited by this intriguing group of parasites. Contrary to expectations, diversification of parasite species appears to be dominated by host-parasite cospeciation (Fahrenholz's rule), and, except for piroplasmids that segregate into Clade VI, host switching is rarely observed. When only domestic mammalian hosts are taken into account, *Babesia* sensu lato (s.l.) parasites of Clades I and II infect only dogs and cats, respectively, *Cytauxzoon* spp. placed into Clade III only infect cats, *Theileria* placed into Clade IV exclusively infect horses, wheras *Theileria* sensu stricto (s.s.)

of Clade V infects only cattle and small ruminants. In contrast, *Babesia* s.s. parasites of Clade VI infect all farm and companion animal species. We outline how the unique ability of transovarial transmission of *Babesia* s.s. piroplasmids of Clade VI facilitates species diversification by host switching to other host vertebrate species. Finally, a deterioration of sequence fidelity in databases is observed which will likely lead to an increased risk of artifactual research in this area. Possible measures to reverse and/or avoid this threat are discussed.

Keywords: Molecular phylogeny; 18S rRNA gene; Host switch; Cospeciation; Piroplasmids; Farm animals; Companion; animals; Cattle; Sheep and goat Horse; Dog; Cat

Introduction

Babesia, *Cytauxzoon*, and *Theileria* are apicomplexan hemoparasites transmitted by ticks to mammals or birds. They belong to the order Piroplasmida, named after the pear-shaped intraerythrocytic parasite stage, the piroplasm (Greek: pera = pear). As other members of the Apicomplexa phylum, they are obligate intracellular parasites and only propagate within host cells. Their life cycle includes a vertebrate as an intermediary host, where only asexual propagation by schizogony and/or merogony takes place, and a tick as the definitive host, where sexual reproduction by gametogony and asexual propagation by sporogony occurs (Uilenberg 2006; Schnittger et al. 2012).

After its transmission by ticks, the infective sporozoite of *Babesia* directly invades erythrocytes, where they propagate by merogony. In contrast, *Theileria* and *Cytauxzoon* sporozoites first invade leukocytes (monocytes, macrophages, or T lymphocytes) and form schizonts. Some schizonts further develop into merozoites and egress from these cells, then invade erythrocytes, where they establish a merogonic propagation cycle (Fig. 1; Kakoma and Mehlhorn 1994; Mehlhorn et al. 1994). Piroplasmids belonging to *Theileria* and *Babesia* sensu lato (s.l.) display transstadial transmission exclusively. In this mode of transmission, the tick first needs to acquire the parasite during the blood meal of the larvae or nymph, which during tick molting episodes is passed on to the subsequent nymph or adult tick stage, respectively (Florin-Christensen and Schnittger 2009). Besides transstadial transmission, the true *Babesia* or *Babesia* sensu stricto (s.s.) parasites evolved the unique ability to invade tick ovaries and eggs, and, subsequently, salivary glands of the tick larvae of the next generation, which is referred to as transovarial transmission. This results in vertical transmission, i.e., the perpetuation of the parasite into the next tick generation. In addition, transplacental transmission has been demonstrated as an alternative form of vertical transmission for an increasing number of piroplasmid species, ensuring parasite progression into the next vertebrate generation without the need for a tick vector (Florin-Christensen et al. 2021).

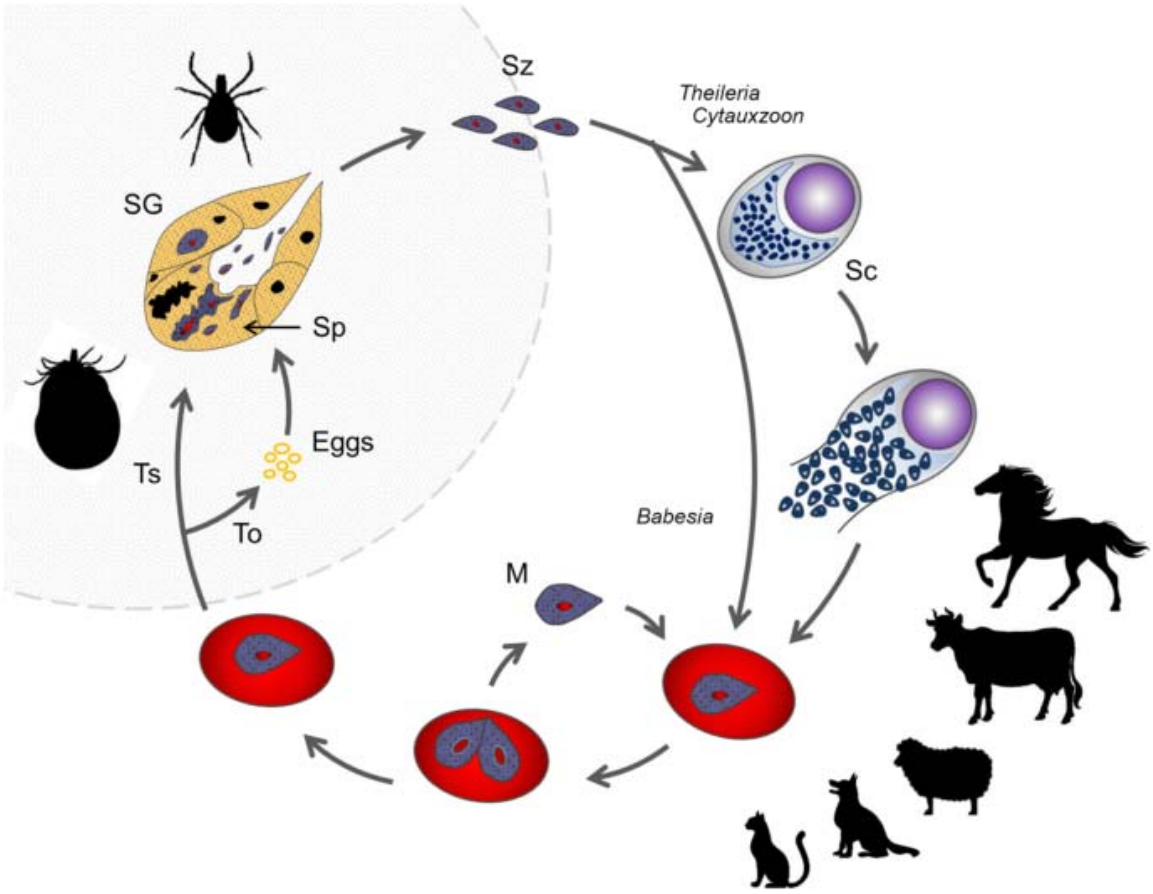

Fig. 1. Schematic representation of the life cycle of piroplasmids. During the blood meal of a piroplasmid-infected tick on a vertebrate host, sporozoites (Sz) are released into the blood with tick saliva. In the case of *Theileria* and *Cytauxzoon*, sporozoites invade leukocytes (macrophages, lymphocytes, or monocytic histiocytes) and divide into intracellular schizonts (Sc). In transforming *Theileria*, the schizont parasite transforms leukocytes into continuously dividing cells that have cancer cell characteristics, metastasizing and invading diverse host cell tissues. In non-transforming *Theileria* or *Cytauxzoon* species, schizonts propagate in leukocytes, but do not transform host cells. Eventually, schizonts egress to invade erythrocytes and develop into merozoites (M), where, depending on the species, they divide either once (formation of duplicated piroplasms) or twice (formation of a Maltese cross), in a process known as merogony. *Babesia* parasites, with the exception of *Rangelia vitalii*, differ from *Theileria* in that tick-transmitted sporozoites directly invade erythrocytes and undergo asexual intraerythrocytic propagation as merozoites. Later intracellular merozoites develop into gametocytes, which do not further propagate. When another tick feeds on an infected vertebrate, gametocyte-infected erythrocytes are ingested and gametocytes egress and develop in *Babesia* into extracellular, ray-shaped isogametes, while in *Theileria*, macro and ray-shaped microgametes are formed. Gametes fuse in the tick midgut and turn into the diploid zygote, or ookinete stage, which invades the gut epithelium. Here, meiosis of the ookinete and subsequent intracellular asexual replication results in the formation of primary kinetes that in the case of *Theileria* directly invade salivary glands (SG) or, in the case of *Babesia*, invade other tick tissues where, through an additional asexual replication, secondary kinetes are generated and invade salivary glands. In salivary glands, metamorphosis of kinetes into sporozoites (Sp) occurs, which are injected into a vertebrate during a blood meal, closing the cycle. In *Babesia* sensu stricto, kinetes can invade the tick ovaries and then eggs, passing into the next tick generation (transovarial transmission, To), in which they develop into sporozoites depending on the species in the larvae or nymph salivary glands. In contrast, *Theileria* and *Babesia* sensu lato pass to the next tick developmental stage after molting (transstadial transmission, Ts) (Jalovecka et al. 2018)

Phylogenetic analysis of piroplasmids based on the 18S rRNA gene has revealed that *Babesia*, *Cytauxzoon*, and *Theileria* represent a polyphyletic assemblage that requires taxonomic revision. Noteworthy, all piroplasmids important for the health of farm and companion animals, as well as those that infect humans, occur within six clades (Clades I to VI, according to Schnittger et al. 2012). Placement in these clades is in some cases accompanied by major life cycle particularities. Thus, the group of true *Babesia* or *Babesia* s.s. species of Clade VI is characterized by transstadial and transovarial transmission, while true *Theileria* or *Theileria* s.s., grouped into Clade V, feature transstadial transmission and a schizont parasite stage (Fig. 2). On the other hand, *T. equi* (Clade IV or Equus clade) displays transstadial transmission and a schizont stage but represents a phylogenetic lineage that is distinct from *Theileria* s.s. and *Babesia* s.s. Likewise, *Cytauxzoon* (Clade III) is characterized by the presence of a schizont stage that infects host cells of the mononuclear reticulohistiocytic system. The remaining piroplasmid lineages can be distinguished from *Babesia* s.s. by exhibiting only transstadial transmission and belong either to Clade I (*B. microti* group) or II (Western clade), and together they are referred to as *Babesia* s.l. (Fig. 2).

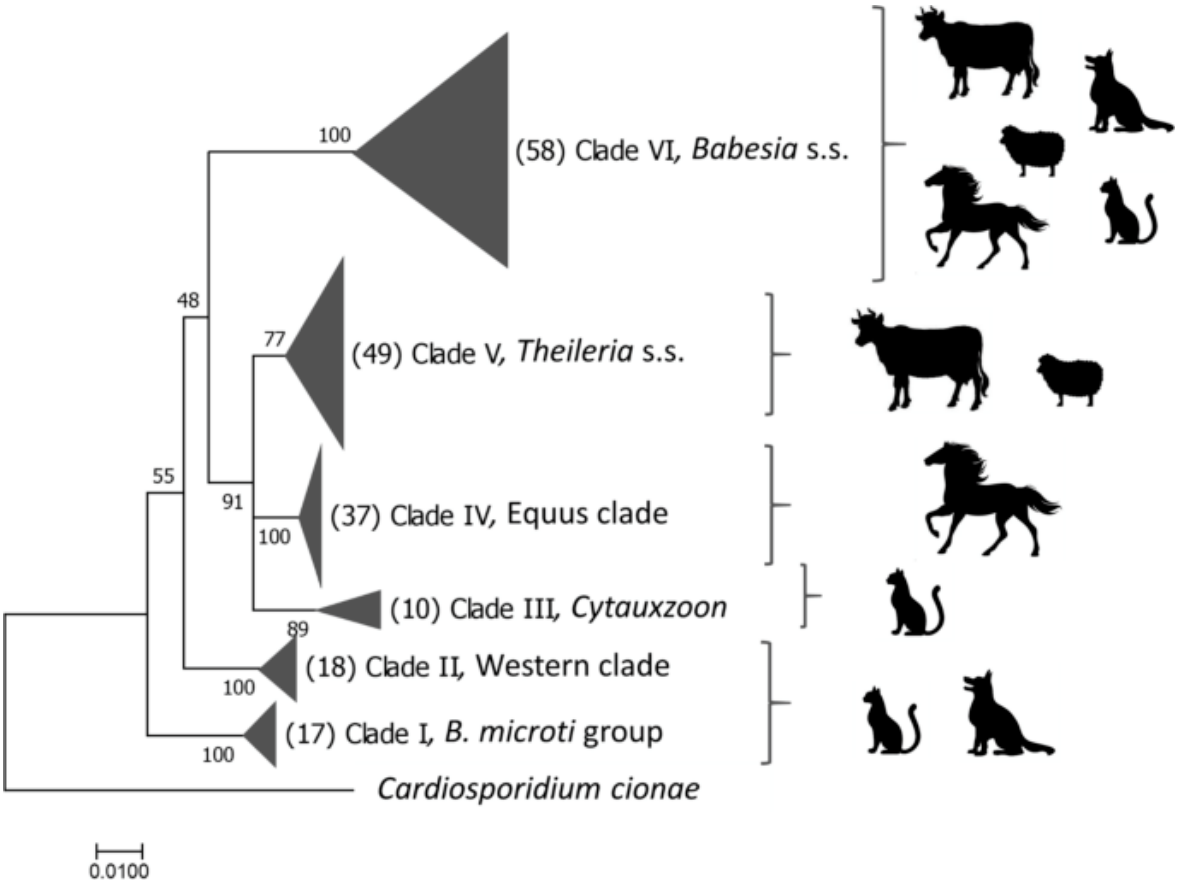

Fig. 2. Molecular phylogenetic tree of 18S rRNA gene sequences of piroplasmid species (Clades I to VI) infecting domestic animals. Tree branches corresponding to Clades I to VI were collapsed due to space limitations. After alignment of 193 nucleotide sequences using MUSCLE, the Kimura 2 ($G + I$) parameter model was applied with a rate difference of 5 categories ($G = 0.48$, $I = 36.50\%$) to infer a maximum likelihood tree (Kimura 1980). For tree estimation, 974 positions were used. The 18S rRNA gene sequence of *Cardiosporidium cionae* (EU052685) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

These six lineages have emerged primarily due to our anthropocentric research orientation and not because of a possibly assumed intrinsic biological precedence. In consequence, at least four additional monophyletic lineages, which are confined to wild mammals (e.g., rhinocerotidae, monotremata, and Australian marsupials) and birds, have been identified and defined, and it is expected that others will follow (Paparini et al. 2012, 2014, 2015; Slapeta et al. 2017; Vanstreels et al. 2015; Yabsley et al. 2017; Greay et al. 2018; Barbosa et al. 2019; Jalovecka et al. 2019; Gonçalves et al. 2021; Ikeda et al. 2021).

This review is focused exclusively on farm and companion animals, and for the reason of clarity and ease of orientation, phylogenetic lineages will be addressed with their original designation as Clades I to VI (Schnittger et al. 2012). However, it is important to be aware that a considerable number of additional phylogenetic lineages exist (Jalovecka et al. 2019).

Different piroplasmid groups have evolved a series of intricate host-pathogen interplay mechanisms to guarantee their efficient propagation. Two different invasion processes of host erythrocytes have been described; *Babesia* s.s. parasite invasion is mediated by the discharge of secretory organelles and the formation of a tight junction between host and parasite membranes, resulting in the transient formation of a parasitophorous vacuole (Yokoyama et al. 2006). In contrast, *Theileria* s.s. parasites use a zipper-like mechanism with no involvement of apical organelles and a parasitophorous vacuole is never formed (Shaw 2003). After invasion, *Babesia* and *Theileria* parasites reside, in contrast to *Plasmodium*, without a parasitophorous vacuole, directly within the cytoplasm of the erythrocyte. The invasion process is very rapid and efficient, ensuring that the parasite quickly finds an intracellular milieu where it can thrive (Sevilla et al. 2018). Some *Babesia* spp. of Clade VI, such as *B. bovis* and *B. caballi*, evolved highly intricate means to escape the host immune defense by antigenic variation, with the participation of large multigene families of surface proteins, and by causing the appearance of adhesive protuberances on the surface of infected erythrocytes that provoke their sequestration in capillaries, avoiding destruction in the spleen (Cooke et al. 2005; Allred 2019). Some *Theileria* species of Clade V, such as *T. annulata*, *T. parva*, and *T. lestoquardi*, are referred to as transforming *Theileria* spp., since they have found a way to propagate by schizont-induced uncontrolled host cell division, synchronizing parasite division with that of their host cell (Ahmed et al. 1999; Mans et al. 2015).

During tick feeding on a competent host, saliva components decrease the host immune response, resulting in both a longer attachment and efficient blood feeding by the tick, contributing to an effective piroplasmid transmission (Maritz-Olivier et al. 2007; Jalovecka et al. 2019). Finally, complex interaction between tick and piroplasmid molecules occurs within tick organs and tissues, which usually allows a balanced parasite propagation with no deleterious consequences for the tick. These complex interactions between ticks, piroplasmids, and their vertebrate reservoirs developed millions of years ago, leading to an equilibrium where the three actors coexist, thus ensuring parasite endurance and dissemination (Florin-Christensen and Schnittger 2009; Penzhorn 2011; Schnittger et al. 2012; Penzhorn et al. 2017).

Human domestication of wild animals led to the development and global expansion of a few animal species that provide food, fiber, work, or company to satisfy human needs. Relatively recently, at least from an evolutionary point of view, ticks and piroplasmids that had coevolved with their natural wild hosts crossed species barriers into domesticated animals. Hence, coadaptation between parasites and their new hosts is not yet completed, and this inadequate coadaptation often leads to severe pathogenicity or even death of infected

animals. Domestic animals currently constitute a staggering 86% of the total biomass of large animals on earth (vs. 16% of wild animals), thus providing a huge ecological habitat for parasites to thrive, propagate, and evolve (Schnittger and Florin-Christensen 2018). Moreover, wild ancestor hosts lived in small, dispersed herds, in relatively confined ecological niches, which limited the geographic distribution of their associated piroplasmid parasites. Contrarily, large numbers of domestic animals are raised in close contact with each other, with different farm species frequently sharing the same pastures. Provided that a competent tick vector is present, this facilitates the transmission of infection of host-specific parasites between individual animals, but also of piroplasmids that are capable of crossing species barriers. The latter is often referred to as accidental infections and may result in severe pathogenicity or, alternatively, in a few propagation cycles within a dead-end host, with still unknown epidemiological relevance (Leemans et al. 1999; Schnittger et al. 2012). Additionally, the close contact between domestic animals and humans promotes the transmission of zoonotic parasites with humans as accidental or dead-end hosts, as is the case for *B. divergens* (Zintl et al. 2003).

Although important progress has been made in piroplasmid research over the last decades, an integrated overview on reported piroplasmid species, isolates, and species complexes infecting farm and companion animals is lacking. This study aims to present a complete compilation of *Babesia*, *Cytauxzoon*, and *Theileria* spp. that are infecting domestic mammals and have been well-characterized using the 18S rRNA or the *cox1* gene as a molecular marker (Supp. data 1 and 2). Piroplasmid taxons are presented according to their phylogenetic classification into Clades I to VI and according to the vertebrate hosts that they infect. On one hand, this allows appreciating the unique characteristics displayed and the diversity of vertebrate hosts infected by each piroplasmid lineage and, on the other hand, provides an insight into the evolutionary relationships that exist between species within each phylogenetic lineage. The approach reveals and anticipates research challenges lying ahead, such as the need of species description of well-defined parasite isolates, a deeper analysis, understanding, and possible resolution of species complexes, and further progress in molecular phylogeny and taxonomy of this group, among others. Furthermore, the comparison of piroplasmid clades results in a more profound understanding of the similarities between and idiosyncrasies of each group within the Piroplasmida, which promotes the formulation of novel research hypotheses.

Piroplasmids in farm animals

Babesia and *Theileria* parasites in cattle

Members of the Bovidae family with productive importance (bovine, *Bos taurus*; water buffalo, *Bubalus bubalis*; zebu, *Bos indicus*; yak, *Bos grunniens*) can undergo infections with several *Babesia* and *Theileria* spp. Some of these parasites cause devastating diseases worldwide with huge economic losses, especially connected to cattle breeding in tropical and subtropical regions. Losses are not only due to animal death, but also to abortion, and losses in meat and milk production. Additional costs are associated with tick control, vaccination, chemotherapy, professional veterinary support, and decreased investment in cattle production in tick-infested areas (Bock et al. 2004; Ganzinelli et al. 2018; Kiara et al. 2018).

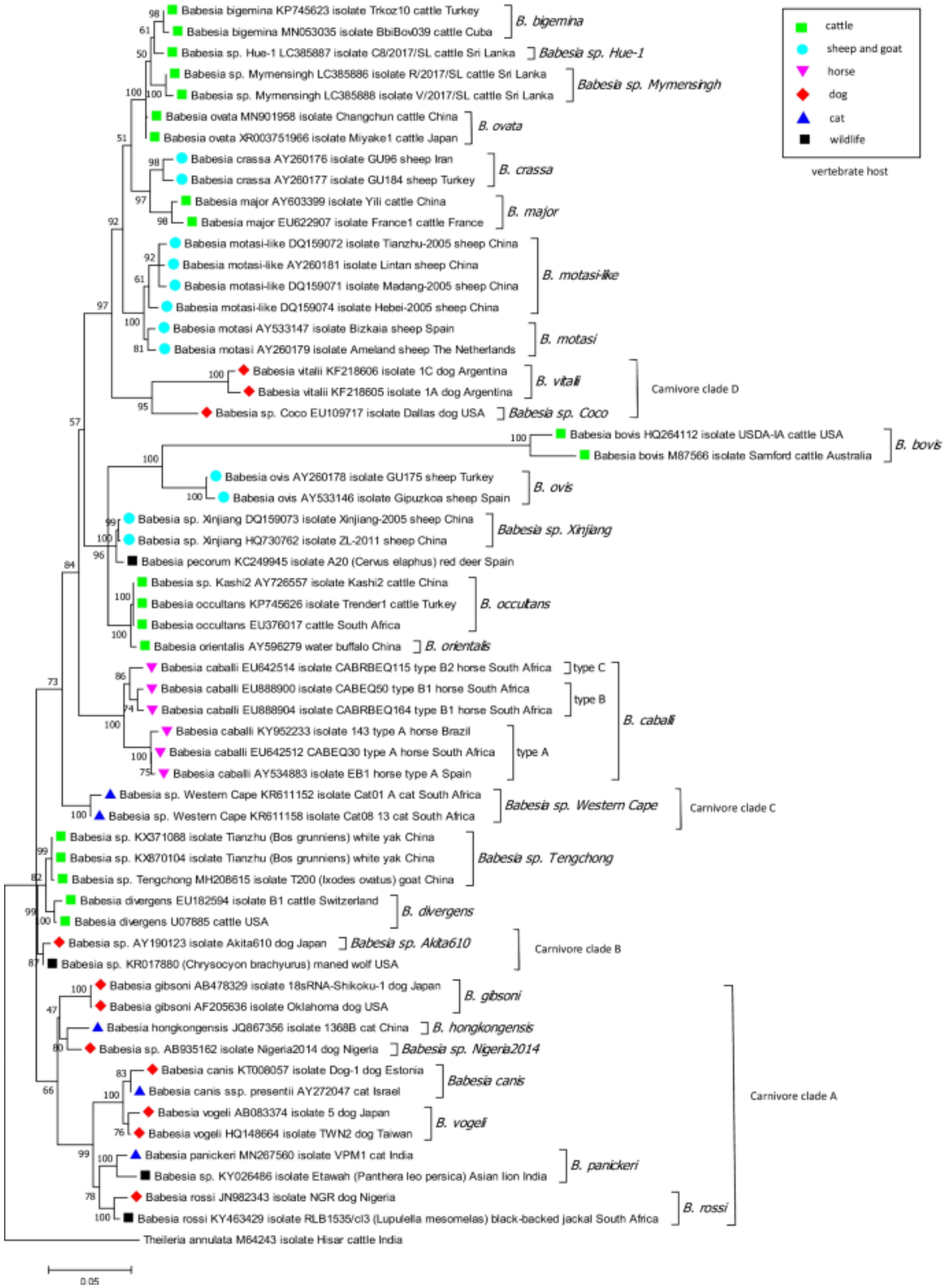

Fig. 3. Molecular phylogenetic tree of 18S rRNA gene sequences of *Babesia* s.s. (Clade VI) infecting domestic animals. Carnivore clades A to D represent subclades of *Babesia* s.s. species that infect carnivore host species. Clades of *B. caballi* type A, B, and C are indicated. After alignment of 57 nucleotide sequences using MUSCLE, the Tamura-Nei ($G + I$) parameter model was applied with a rate difference of 5 categories ($G = 0.14$, $I = 39\%$) to infer a maximum likelihood tree (Tamura and

Nei 1993). For tree estimation, 1227 positions were used. The 18S rRNA gene sequence of *T. annulata* (M65263) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

All ruminant-infecting *Babesia* species belong to the sensu stricto group (corresponding to Clade VI, Schnittger et al. 2012), which are characterized by the lack of a schizont stage, asexual reproduction exclusively within red blood cells in vertebrate hosts, and the occurrence of transovarial transmission in the tick vector (Uilenberg 2006; Schnittger et al. 2012; Jalovecka et al. 2019) (Table 1, Figs. 2 and 3). Among *Babesia* species that infect *Bos taurus* cattle, *B. bovis* and *B. bigemina* have the highest impact on animal health and productivity, while *B. divergens*, geographically more restricted, is relevant as a cattle and human pathogen. In *B. bovis* infections of naïve bovines, sequestration of infected erythrocytes in the microvasculature of the brain and lungs can provoke cerebral babesiosis and respiratory distress syndrome, respectively, and results in low levels of parasitemia (Cooke et al. 2005; Ganzinelli et al. 2018). On the other hand, acute babesiosis caused by *B. bigemina* and *B. divergens* progress with high parasitemia, and pathogenicity is mainly associated with massive erythrocyte destruction leading to severe anemia. Hemolysis-associated hemoglobinuria is observed in acute *B. bigemina* and *B. divergens* cases and in the late clinical stages of *B. bovis* infections, for which bovine babesiosis has received the common name of "redwater." In addition, fever is frequent in infections by these three parasites, which can lead to abortions in pregnant cattle and temporary reduction of fertility in bulls. Death may occur if animals are not treated (Zintl et al. 2003; Bock et al. 2004; Henker et al. 2020).

Human *Babesia divergens* infections can be asymptomatic and self-limiting in healthy individuals or be accompanied by fever, chills, headache, fatigue, loss of appetite, nausea, and shortness of breath. However, in immunocompromised, elderly, and splenectomized patients, infections can lead to severe clinical signs, including hemolytic anemia, splenomegaly, hepatomegaly, and renal failure, and are frequently fatal when untreated (Young et al. 2019).

Babesia bovis and *B. bigemina* are transmitted by the ixodid one-host ticks *Rhipicephalus microplus*, *R. annulatus*, *R. australis*, and *R. geigyi*, found in tropical and subtropical regions of the world (Gray et al. 2019; Estrada-Peña et al. 2012). Furthermore, *B. bigemina* can be transmitted by *R. decoloratus* and *R. evertsi*, which extends the distribution of this parasite in the African continent, making it the most widespread bovine *Babesia* species (Rodriguez et al. 2013). *Babesia divergens* is transmitted by *Ixodes ricinus* in Europe and Northern Africa. This tick thrives in moisture-saturated microhabitats and tolerates a large range of temperatures, which determines its distribution in various climatic regions (Zintl et al. 2003; Azagi et al. 2021).

Recently, a yet unnamed novel species, *Babesia* sp. Mymensingh, causing clinical babesiosis characterized by fever, hemoglobinuria, anemia, and jaundice, has been identified in cattle of Vietnam, Sri Lanka, Uganda, Mongolia, and Argentina. This species is most closely related to *B. bigemina* (Fig. 3). The tick vector of this parasite is still unknown (Roy et al. 2018; Sivakumar et al. 2018; Otgonsuren et al. 2020; Sivakumar et al. 2020). Identification of an additional isolate designated *Babesia* sp. Hue-1 warrants further characterization to confirm its status as species (Sivakumar et al. 2018).

Table 1 *Babesia* and *Theileria* spp. of cattle and water buffalo

Species	Tick vector	Geographical distribution	Pathogenicity	Clade	Reference
B. bovis	*Rhipicephalus microplus*, *R. annulatus*, *R. geigyi*, *R. australis*	Africa, America, Asia, Australia, Europe	Fever, anemia, hemoglobinuria, cerebral babesiosis, and respiratory distress syndrome	*Babesia* s.s. (Clade VI)	Bock et al. 2004; Uilenberg 2006; Schnittger et al. 2012; Estrada-Peña et al. 2012; Florin-Christensen et al. 2014
B. bigemina	*R. microplus*, *R. annulatus*, *R. geigyi*, *R. decoloratus*, *R. evertsi*		Fever, anemia, and hemoglobinuria		
B. divergens	*Ixodes ricinus*, *I. persulcatus*	Europe, Northern Africa	Fever, anemia, and hemoglobinuria		Zintl et al. 2003; Azagi et al. 2021
B. ovata	*Haemaphysalis* sp.	Asia	Low pathogenicity		Sivakumar et al. 2016
B. major	*Haemaphysalis* sp.	Asia, Europe	Low pathogenicity		Bock et al. 2004; Schnittger et al. 2012
B. occultans	*Hy. marginatum*	Africa, Europe	Low pathogenicity		Blouin and van Rensburg 1988; Decaro et al. 2013
B. orientalis	*R. haemaphysaloides*	Asia	Fever, anemia, icterus, hemoglobinuria		Liu et al. 1997; He et al. 2017
Babesia sp. Mymensingh	nk	Vietnam, Sri Lanka, Uganda, Mongolia, Argentina	Fever, hemoglobinuria, anemia, and jaundice		Sivakumar et al. 2020; Roy et al. 2018
Babesia sp. Tengchong	*Ixodes ovatus*	China	Asymptomatic		Li et al. 2020
T. annulata	*Hyalomma* sp.	North Africa, Sudan, Middle East, Ethiopia, central and western Asia	Tropical theileriosis: fever, hemorrhages, anemia, and jaundice	*Theileria* s.s. (Clade V)	Sivakumar et al. 2014
Theileria sp. Yokoyama	nk	Sri Lanka	nk		Sivakumar et al. 2019
T. parva	*Rhipicephalus appendiculatus/R. zambeziensis*	East, central, and southern Africa	East Coast fever		Agina et al. 2020
T. taurotragi	*Rhipicephalus* sp.	Eastern, southern, and central Africa	Considered non-pathogenic, but recently pathogenicity has been observed		Sivakumar et al. 2014; Biasibetti et al. 2016
T. orientalis (syn. *T. buffeli/T. sergenti/T. sinensis*)	*Haemaphysalis* sp.	Worldwide	Oriental theileriosis exclusively caused by the Ikeda and Chitose types		Watts et al. 2016
T. mutans	*Amblyomma* sp.	Africa, Caribbean Islands	Non-pathogenic		Sivakumar et al. 2014
T. verifera	*Amblyomma* sp.	Sub-Saharan Africa	Non-pathogenic		Kiara et al. 2018
Theileria sp. MSD	nk	South Africa, Uganda	Non-pathogenic		Chae et al. 1999; Byaruhanga et al. 2016

nk, not known

Besides *Bos taurus* infections, *B. bovis* and *B. bigemina* commonly cause asymptomatic infections in zebu (*Bos indicus*) and water buffalo (*Bubalus bubalis*) (Ferreri et al. 2008; Jonsson et al. 2008; Romero-Salas et al. 2016; Benitez et al. 2018). In addition, water buffaloes from Vietnam and Sri Lanka were also found to be infected with *Babesia* sp. Mymensingh (Sivakumar et al. 2020). Absent or attenuated clinical signs upon infection of these ruminants might have resulted from long coevolutionary adaptations with *Rhipicephalus* ticks and *Babesia* parasites. Their natural resistance to hemoparasites may be one of the reasons why these types of bovids are favored for meat and/or milk production in many tick-endemic regions (Jonsson et al. 2008; Benitez et al. 2012, 2018; Florin-Christensen et al. 2014).

Babesia spp. infecting cattle with low virulence and limited geographic distribution include *B. ovata* and *B. major*, transmitted by *Haemaphysalis* sp. (Ganzinelli et al. 2018). However, in the case of *B. ovata*, immunocompromised cattle or those coinfected with *T. orientalis* can develop clinical disease (Sivakumar et al. 2016). Phylogenetic analysis strongly suggests that *Babesia* sp. Kashi, isolated in China from *Hyalomma anatolicum* ticks and causing asymptomatic infections, is identical to *B. occultans* (Fig. 3, Luo et al. 2002, 2005). *Babesia occultans*, reported to be transmitted by *Hyalomma* sp., has generally been regarded as mildly pathogenic. Nonetheless, acute bovine babesiosis cases associated with this parasite have been reported in Italy and Iran indicating that this parasite may be of greater clinical relevance than initially thought (Decaro et al. 2013; Noaman et al. 2021). *Babesia orientalis* is closely related to *B. occultans*, transmitted by *R. haemaphysaloides*, and has low virulence in cattle; however, water buffaloes are highly susceptible to this parasite and clinical cases with fever, anemia, icterus, hemoglobinuria, and even death have been reported in Asia (Liu et al. 1997; He et al. 2017).

Babesia bigemina, *B. bovis*, and *B. ovata* infections were also detected in yaks (*Bos grunniens* and *Bos mutus*), long-haired bovids adapted to high altitudes and important for the livelihood and economy of dwellers of the Himalayan region. The relevance of these infections for the welfare and productivity of yaks is still unknown (Li et al. 2018). Importantly, an additional novel species, *Babesia* sp. Tengchong, has been isolated from white yaks and *Ixodes ovatus* ticks feeding on goats (Fig. 3, Liu et al. 2017; Li et al. 2020). The significance of goats as a host of this species needs to be confirmed.

Finally, infections of cattle with non-bovine *Babesia* spp., such as the ovine *B. motasi*, are occasionally detected by molecular methods. However, it is unclear whether these parasites can efficiently use bovines as intermediary hosts (Sun et al. 2020).

Unlike naïve adult cattle, young animals do not generally present clinical signs upon *Babesia* sp. infection due to the existence of competent innate immune defense mechanisms (Rodriguez et al. 2013). Animals that survive acute babesiosis develop a strong protective adaptive immunity and do not manifest clinical signs. However, low-level persistent infections allow parasite transmission and perpetuation in tick-endemic areas (Bock et al. 2004).

In summary, the clinical manifestations of *Babesia* sp. infections of bovids depend on the pathogenic phenotype of the infecting species and strain but host factors, such as species, breed, age, and immune status, are also involved (Chauvin et al. 2009). While the impact of clinical babesiosis is clear, it remains to be investigated whether asymptomatic infections of bovids with *Babesia* spp. result in a decrease in health and/or productivity parameters.

Distinct management strategies, including tick control using acaricides, chemotherapy, and vaccination, are applied to prevent or decrease the impact of clinical manifestations of bovine babesiosis in endemic areas. Although chemical acaricides are still the main means of tick control, they do have important shortcomings, such as contamination of the environment and animal products with chemical residues and the emergence of acaricide-resistant ticks (Maya-Delgado et al. 2020; Kumar et al. 2020). An environment-friendly and efficacious alternative to acaricides to reduce tick infestation consists of immunization of cattle with recombinant tick antigens. This strategy has resulted in the development of commercial vaccines based on the *R. microplus* Bm86 gut antigen, as well as a great deal of research efforts to identify additional tick targets for new anti-tick vaccine formulations (de la Fuente et al. 2007; Ndawula and Tabor 2020).

Imidocarb dipropionate and diminazene acetate are the most common drugs used in the treatment of babesiosis. However, the accumulation of drug residues in meat and milk and the risk of development of drug resistance have prompted an active search for alternative suitable chemotherapeutics (Mosqueda et al. 2012; Rodriguez et al. 2013).

Live vaccines based on attenuated parasites are available in some countries for the prevention of *B. bovis* and *B. bigemina* infections and are effective against the devastating effects of the acute disease. However, although attenuated strains are usually poorly virulent for young calves, they can cause severe acute babesiosis and death when applied to adult animals (Florin-Christensen et al. 2014, 2021). Thus, there is considerable interest in developing alternative options based on recombinant subunit vaccines. Genome analysis, transfection systems, and gene-editing technology can highly benefit and accelerate the development of such novel vaccine strategies (Silva et al. 2018; Suarez et al. 2018).

Theileria sp. parasites that infect bovids belong to a monophyletic group corresponding to Clade V as defined in Schnittger et al. (2012) (Table 1, Figs. 2 and 4). Members of this clade are characterized by presenting a schizont stage in lymphoid cells and piroplasms in red blood cells of the vertebrate host, and exclusive transstadial but not transovarial transmission in the tick (Kiara et al. 2018).

Fig. 4. Molecular phylogenetic tree of 18S rRNA gene sequences of *Theileria* s.s. (Clade V) infecting domestic animals. After alignment of 44 nucleotide sequences using MUSCLE, the Tamura-Nei (*G* + *I*) parameter model was applied with a rate difference of 5 categories (*G* = 0.10, *I* = 72.50%) to infer a maximum likelihood tree (Tamura and Nei 1993). For tree estimation, 1378 positions were used. The 18S rRNA gene sequence of *Cytauxzoon felis* (AY679105) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016) The clade of ´transforming *Theileria*´ refers to piroplasmid species that invade and transform leukocyte host cells

Among bovine-infecting *Theileria* parasites, *T. annulata*, *T. parva*, and *T. orientalis*, etiological agents of tropical theileriosis, East Coast fever (ECF), and oriental theileriosis, respectively, are the most important species from an economic point of view (Kiara et al. 2018).

Some *Theileria* parasites of Clade V (*T. annulata*, *T. parva*, *T. taurotragi*, possibly the recently in bovids identified *Theileria* sp. Yokoyama and the ovine-infecting species *T. lestoquardi*) have acquired the ability to transform leukocytes of their hosts (Sivakumar et al. 2014, 2019). The transforming *Theileria* species are contrasted with non-transforming species (*T. orientalis*, *T. mutans*, and *T. velifera* in bovids). The former possess the ability to invade leukocytes, develop into schizonts, and synchronize schizont division with that of the host cells they infect. Transformed leukocytes proliferate indefinitely and disseminate in infected cattle in a cancer-like style. This unique feature results in a complex interplay between parasite and host and is often associated with high pathogenicity and mortality (Mans et al. 2011; Tajeri et al. 2021). Under the supposition of parasite-host cospeciation, phylogenetic inference supports the view that the ability of host cell transformation has been acquired only once by the most recent common ancestor (MRCA) of this monophyletic group about 14 to 16 mya (Fig. 4, Hassanin et al. 2012; Pienaar et al. 2018; Jalovecka et al. 2019). This highlights that host cell transformation is an autapomorphy and therefore not suitable to define the *Theileria* s.s. group.

Theileria annulata is probably the most important ruminant-infecting *Theileria* species with an estimation of over 250 million cattle heads at risk. Its distribution extends from North Africa through Southern Europe and the Middle East into India, southern regions of Russia, and Central Asia. It infects cattle as well as water buffalo, its likely natural host, and is transmitted by *Hyalomma* ticks (Sivakumar et al. 2014; Kiara et al. 2018; Gharbi et al. 2020). *Theileria annulata* sporozoites invade MHC class II–expressing cells, mainly macrophages and B cells, leading to the formation of schizonts and uncontrolled proliferation of parasite-infected host cells. Further multiplication of the piroplasms occurs in the red blood cells, and high parasitemia levels are associated with hemolytic anemia. In addition, tropical theileriosis is characterized by enlarged lymph nodes, fever, increased pulse and respiratory rates, swollen eyelids, profuse lachrymation, jaundice, and sometimes death (Agina et al. 2020). A *T. annulata*-like parasite was recently detected in cattle from Sri Lanka, and phylogenetic analysis based on the 18S rRNA and cytochrome b gene showed that it corresponds to a new species, which has been provisionally named *Theileria* sp. Yokoyama (Fig. 4). Morphological, clinical, and pathological differences of this parasite with respect to *T. annulata* remain to be studied (Sivakumar et al. 2019). However, as this species segregates into the monophyletic group of the transforming *Theileria*, it is reasonable to assume that it shares this characteristic with the other *Theileria* species of this group (see above).

Theileria parva poses an enormous economic impact, especially on pastoralist and small farmers in Eastern and Southern Africa. It is mainly transmitted by the widespread tick vector *R. appendiculatus*, as well as by *R. zambeziensis* (Morrison et al. 2015, 2020). *Theileria parva* transformation of T cells is associated with high virulence. ECF clinical signs include anorexia, fever, leukopenia, and enlarged lymph nodes. In addition, diarrhea, nasal discharge, soft coughs, and difficulties in breathing due to the accumulation of fluid in the lungs may occur. Sometimes, severe congestion and hemorrhages in the meninges and in the brain accompanied by nervous signs, paralysis, and death can take place if the parasites invade the central nervous system, in a manifestation known as bovine cerebral theileriosis or turning sickness (Morrison et al. 2015; Kiara et al. 2018).

Based on molecular detection in different potential hosts and in vitro experiments of host cell invasion, the African buffalo (*Syncerus caffer*) is considered to be the natural reservoir of *T. parva* (Pienaar et al. 2018; Morrisson et al. 2020). Tick transmission of *T. parva* isolates from buffalo causes high mortality in cattle, a sickness termed Corridor disease. Strains adapted to

cattle (cattle-derived *T. parva*) can be transmitted between cattle by ticks, causing ECF or January disease, and form a separately maintained subset population of those found in buffalo (Morrison et al. 2020). These observations highlight the adaptation and/or selection process undergone by the *T. parva* parasite population of African buffalo enabling the use of cattle as hosts after these bovids were introduced into the African continent. This may be an ongoing process taking place when both host animal species are maintained in proximity (Uilenberg 1999; Sivakumar et al. 2014). Consistent with this notion, African buffalo-derived strains were found to have a higher genetic diversity, with double the number of variants, compared to cattle-derived strains (Maboko et al. 2021).

Theileria taurotragi is transmitted by *R. appendiculatus* and, accordingly, shows an overlapping distribution with *T. parva*. Although this parasite is generally considered mildly pathogenic, cerebral theileriosis cases observed in bovine and zebu cattle have been linked to *T. taurotragi* infections (De Vos et al. 1981; Mans et al. 2015; Biasibetti et al. 2016). The natural reservoirs of *T. taurotragi* are the Tragelaphini species nyala (*Tragelaphus angasii*), eland (*Taurotragus oryx*), and kudu (*Tragelaphus strepsiceros* and *T. imberbis*), while this parasite does not infect the African buffalo (Pienaar et al. 2018). The Tragelaphini are a sister taxon of the Bovini (comprising of Bovina and Bubalina) within the Bovinae subfamily. Accordingly, assuming parasite-host cospeciation, *T. taurotragi* and Tragelaphini represent sister species to the remaining transforming *Theileria* spp. and Bovini hosts, respectively, except for sheep infected by *T. lestoquardi* in which host switching from cattle to sheep has occurred (Schnittger et al. 2003; Hassanin et al. 2012; Pienaar et al. 2018; Jalovecka et al. 2019).

Among the bovid non-transforming *Theileria* spp., *T. orientalis* is the most relevant and has a worldwide distribution with its greatest economic impact recorded in Australia, Japan, and New Zealand. Acute cases of oriental theileriosis, characterized by marked anemia as the main clinical sign, as well as pyrexia and elevated heart and respiratory rates, appear where naïve cattle have been introduced into an endemic area or in animals under stress (Watts et al., 2016).

Two additional related species, defined according to their geographical origin, *T. sergenti* in Japan and *T. buffeli* in Australia, were later reclassified as *T. orientalis*, based on the sequence analysis of the 18S rRNA gene and the major piroplasm surface protein (MPSP) genes (Kamau et al. 2011; Sivakumar et al. 2014; Watts et al. 2016). Analysis of MPSP sequences also revealed the presence of twelve MPSP types currently, some of which correlate with virulence (Li et al. 2020; Gebrekidan et al. 2020). Thus, the Ikeda (type 2) and Chitose types (type 1) are associated with anemia, while the Buffeli type (type 3), type C (type 4), and other isolates are not (Eamens et al. 2013; Sivakumar et al. 2014; Jenkins et al. 2015). A recent phylogenomic study suggests that Ikeda may most likely represent a different species when compared to Chitose and Buffeli types (Bogema et al. 2018). Furthermore, the third group of closely related isolates from China, referred to as *T. sinensis*, group within the *T. orientalis* complex. Consequently, this suggests that the name *T. sinensis* can be considered obsolete and these isolates should be referred to as *T. orientalis* (Fig. 4). Combined infections of cattle with different *T. orientalis* genotypes have been suggested to contribute to the persistence of the parasite in the host by simultaneously presenting several targets to its immune system (Eamens et al. 2013). Asymptomatic infections of water buffaloes and yaks with *Theileria orientalis* have also been reported (Sivakumar et al. 2014; Li et al. 2018).

Other non-transforming *Theileria* spp. described in cattle that are often found in high prevalence and are not associated with clinical cases are *T. mutans* and *T. velifera*, which are transmitted by *Amblyomma* ticks (Sivakumar et al. 2014; Byaruhanga et al. 2016; Kiara et al. 2018 Ouedraogo et al. 2021). The *T. mutans* reference isolate (AF078815, cattle, Intona, Kenya) groups with isolates from zebu and goat into a single clade that is most closely related to *Theileria* sp. MSD, a species that has been isolated from cattle, zebu, African buffalo, and goats (Fig. 4, Byaruhanga et al. 2016; Chatanga et al. 2021). Importantly, isolates exclusively identified in African buffalo and referred to as *T. mutans*-like can be clearly distinguished from *T. mutans* and *Theileria* sp. MSD identified in cattle, zebu, and goat (Chaisi et al. 2013; Byaruhanga et al. 2016). Additional studies may be necessary to delineate the additional isolates that are associated with *T. mutans*.

Bovine theileriosis can be partially controlled by acaricides to decrease tick infestation and, to a lesser extent, by the production of cross breeds of cattle that are more resistant to the parasite than European breeds, as well as the use of anti-theilerial drugs to treat clinical cases. However, concerning the latter approach, several cases of treatment failure have been reported for *T. annulata* (Gharbi et al. 2020). Protection against clinical signs associated with acute tropical theileriosis can be achieved by vaccination with attenuated macroschizont cell lines, but so far this approach has only been experimental (Morrison and McKeever 2006). In the case of ECF, the so-called infection and treatment method is currently the only preventive immunization protocol available. It involves the inoculation of three different strains of live *T. parva* sporozoites, together with the administration of long-acting oxytetracycline (Morrison et al. 2015; Agina et al. 2020). As mentioned above for bovine babesiosis, preventive protocols based on live parasites imply a cumbersome production and the risk of coinfection with other pathogens. Important research efforts have been concentrated on identifying potentially useful vaccine antigens for subunit vaccines, but the experimental trials carried out so far in cattle did not yield satisfactory results (Musoke et al. 2005; Fry et al. 2019; Florin-Christensen et al. 2021).

Babesia and *Theileria* in sheep and goats

Piroplasmids of veterinary importance infecting sheep and goats all belong to *Babesia* s.s. (Clade VI) and *Theileria* s.s. (Clade V) (Table 2, Figs. 2, 3, and 4). The diversity of these piroplasmids is challenging to assess from literature for several reasons, including (i) some species were described before the onset of molecular taxonomy and in some cases it is not possible to know to which species these old reports refer to; (ii) species names have changed since they were first described; (iii) publications are wrongly cited; (iv) deposited 18S rRNA sequences are too short to warrant a reliable phylogenetic analysis; or (v) sequences deposited in databases are not accompanied by a descriptive publication. In addition, several sequence variants of the 18S rRNA gene have been defined, but it is not yet clear if they belong to different species or subspecies. Despite these constraints, this section aims to provide a concise and thorough overview of what is currently known about small ruminant piroplasmids.

Table 2 *Babesia* and *Theileria* spp. of sheep and goat

Species	Tick vector	Geographical distribution	Pathogenicity	Clade	Reference
B. ovis	*Rhipicephalus bursa*, *Rhipicephalus turanicus*	Southern Europe, Middle East, some African and Asian countries	Pathogenic for adult sheep, non-pathogenic for goat	*Babesia* s.s. (Clade VI)	Babes 1892; Yeruham et al. 1998
B. crassa	nk	Iran, Turkey	Mildly pathogenic		Hashemi-Fesharki and Uilenberg 1981
B. motasi	*Haemaphysalis punctata*	Europe	Mildly pathogenic for sheep, severely pathogenic for goats		Uilenberg et al. 1980; Lewis et al. 1981
B. motasi-like (*Babesia* sp. Lintan)	*Haemaphysalis* sp., *Dermacentor silvarum*, *Ixodes persulcatus*, *Rhipicephalus sanguineus* s.l.	China	Moderately to severely pathogenic		Liu et al. 2007; Niu et al. 2016
Babesia sp. Xinjiang	*Haemaphysalis longicornis*, *Hyaloma anatolicum*	China	Moderately pathogenic		Liu et al. 2007; Niu et al. 2017
T. uilenbergi	*Haemapysalis qinghaiensis*, *Haemaphysalis longicornis*	China	Highly pathogenic for sheep and goats resulting in important mortality	*Theileria* s.s. (Clade V)	Schnittger et al. 2000; Schnittger et al. 2003; Yin et al. 2007
T. luwenshuni	*Haemapysalis qinghaiensis*				
Theileria sp. OT1 (syn. *T. luwenshuni*)	nk	Spain	Non-pathogenic		Nagore et al. (2004a, b)
T. lestoquardi (syn. *T. hirci*)	*Hyalomma anatolicum anatolicum*, *Rhipicephalus sanguineus* s.l.	Sudan, Egypt, India, Turkey, Iran, Saudi Arabia, and Oman	Malignant sheep theileriosis: fever, lethargy, cough, lymphadenopathy, weight loss, high mortality		Hooshmand-Rad and Hawa 1973a, b
T. ovis	*Hyalomma* sp., *Rhipicephalus evertsi*	Europe, Sudan	Non-pathogenic		Leemans et al. 1997; Uilenberg 1981; Schnittger et al. 2003
Theileria sp. Malawi	nk	Malawi	Non-pathogenic		Chatanga et al. 2021
T. separata	*Rhipicephalus evertsi*	Tanzania, Kenya, China?	Non-pathogenic		Uilenberg and Andreasen 1974
Theileria sp. OT3	nk	Spain, Turkey	Non-pathogenic (but increased rate of abortion was observed)		Nagore et al. 2004a, b; Altay et al. 2007
Theileria sp. MK	nk	Turkey	Non-pathogenic		Altay et al. 2007

nk, not known

The first description of an intraerythrocytic microorganism causing disease in sheep was carried out by Viktor Babes in Romania soon after his first similar observation for bovines (Babes 1892). This pathogen, later named *Babesia ovis*, is now known to infect sheep and goats in Southern Europe, the Middle East, and some African and Asian countries, where it is transmitted by *Rhipicephalus bursa* and *R. turanicus* (Yeruham et al. 1998; Yin and Luo 2007; Ranjbar-Bahadori et al. 2012; Rjeibi et al. 2014). While infections of young animals are normally asymptomatic, adult naïve sheep can experience a severe disease with fever, hemolytic anemia, hemoglobinuria, and icterus, as well as significant mortality (Hurtado et al. 2015; Sevinc et al. 2013). Contrarily, there are no reports on clinical cases in goats infected with *B. ovis*. Two additional *Babesia* species have long been associated with infections of small ruminants: *B. crassa*, isolated in Iran, with low pathogenicity and unknown vector, and *B. motasi*, initially isolated in Europe, and transmitted by *Haemaphysalis* ticks. *Babesia motasi* has been suggested to comprise more than one species or subspecies, differing in pathogenicity, infectivity to sheep and goats, and morphology (Lewis and Herbert 1980; Uilenberg et al. 1980; Lewis et al. 1981; Uilenberg 2006). Infections with *B. motasi* lead to mild clinical signs in sheep but can result in severe anemia, ill thrift, and death in goats (Smith and Sherman 2009).

Several Chinese *Babesia* isolates from small ruminants, including *Babesia* sp. BQ1 (Lintan), *Babesia* sp. BQ1 (Ningxian), *Babesia* sp. Tianzhu, *Babesia* sp. Madang, *Babesia* sp. Hebei, and *Babesia* sp. Liaoning, were assigned to a *B. motasi*-like phylogenetic group, due to their similarities in morphology and 18S rRNA sequence to a European *B. motasi* isolate, as well as being also transmitted by *Haemaphysali*s sp. ticks (Fig. 3, Liu et al. 2007; Niu et al. 2016). In several publications, these parasites from China are referred to as *B. motasi*, but in this review, they are named *B. motasi*-like to avoid confusion. The finding of *B. motasi*-like DNA in *Dermacentor silvarum*, *Haemaphysalis quinghaiensis*, *Haemaphysalis longicornis*, *Ixodes persulcatus*, and *Rhipicephalus sanguineus* ticks from different areas of China might indicate an increased range of suitable vectors for these parasites and requires further investigation (Niu et al. 2016). The *B. motasi*-like group separates into two clades upon analysis of apicoplast and mitochondrial genomes, on one hand, into *Babesia* sp. Lintan and *Babesia* sp. Tianzhu, and, on the other hand, into *Babesia* sp. Hebei and *Babesia* sp. Ningxian, each of which may correspond to two distinct species or subspecies (Wang et al. 2019, 2020).

An additional *Babesia* genotype, transmitted by *Hyalomma anatolicum* and *Haemaphysalis quinghaiensis* ticks and causing subclinical infections, was discovered in sheep in Xinjiang, China (Liu et al. 2007; Guan et al. 2009). It received the name *Babesia* sp. Xinjiang and could be clearly differentiated from the *B. motasi*-like phylogenetic group by its 18S rRNA sequence, as well as analysis of its apicoplast and mitochondrial genomes, indicating it is a species on its own (Guan et al. 2016; Wang et al. 2019, 2020). Detection of *Babesia* sp. Xinjiang DNA in questing *Haemaphysalis longicornis* ticks opens the possibility that this is an additional vector for this piroplasmid (Niu et al. 2017). Interestingly, phylogenetic comparison of 18S rRNA genes shows that *Babesia* sp. Xinjiang is closely related to *B. pecorum*, described from cervids in Spain, and to *Babesia* species isolated from giraffe in South Africa. However, the far distant non-overlapping geographic distribution of these isolates and infection of distantly related host species distinguish them as different species (Jouglin et al. 2014).

Recently *B. venatorum* has been detected in sheep in the UK. In Continental Europe, roe deer (*Capreolus capreolus*) is considered the natural host of this species. Whether this represents an accidental infection, or whether the parasite is well established in this host, remains to be

confirmed (Gray et al. 2019). *Babesia taylori* and *B. foliate* have also been reported to infect sheep and goats; however, as no molecular data are available for these species, it is not possible to verify their identity and taxonomic position (Levine 1985).

Ovine theileriosis is of concern for small ruminant producers in different regions of the world. In addition to direct costs due to morbidity and mortality, the high mortality of naïve animals after their first exposure to ticks prevents crossbreeding of indigenous animals with breeds imported from tick-free areas, therefore hampering the possibility of genetic improvement of herds from tick-endemic areas (Mehlhorn et al. 1994; Yin et al. 2002). Several *Theileria* species have been described to infect small ruminants, including *T. lestoquardi*, *T. uilenbergi*, *T. luwenshuni*, *T. ovis*, and *T. separata* (Ahmed et al. 2006). Additionally, *Theileria* sp. isolates that represent yet unrecognized species have been reported (Table 2, Figs. 2 and 4).

Theileria lestoquardi, initially named *T. hirci*, is highly pathogenic and belongs, together with *T. annulata*, *T. parva*, and *T. taurotragi* to the transforming group of *Theileria* spp., a unique characteristic proposed to derive from a common ancestor of this group as outlined previously (see "*Babesia* and *Theileria* parasites in cattle"; Hooshmand-Rad and Hawa 1973a; Schnittger et al. 2003; Sivakumar et al. 2014). However, phylogenetic inference suggests that in contrast to the other transforming *Theileria* spp. which have most likely emerged by cospeciation evolution, *T. lestoquardi*, which is very closely related with *T. annulata*, has evolved most recently following a host switch to sheep. This view is supported by the fact that both piroplasmids, *T. annulata* and *T. lestoquardi*, are transmitted by the same tick species (Schnittger et al. 2003). Uncontrolled proliferation of *T. lestoquardi* schizonts within lymphocytes results in malignant theileriosis with high morbidity and mortality rates in sheep (El Iman et al. 2015; Tageldin et al. 2005). Interestingly, cross-infectivity studies show that acute infections with this parasite are characterized by generalized enlargement of the superficial lymph nodes, high fever, cessation of rumination, diarrhea or constipation, jaundice, and hemorrhages, while chronic infections are accompanied by intermittent fever, inappetence, anemia, jaundice, and emaciation (Tageldin et al. 2005). The outcome depends on genetics, nutrition, concomitant diseases, and infection dose (Preston 2001; El Imam et al. 2015). Although goats usually undergo subclinical infections with *T. lestoquardi*, an outbreak confirmed to be caused by this parasite, with typical clinical manifestations of malignant theileriosis and high lethality, was reported in a herd of goats in Sudan (Taha et al. 2011). *Theileria lestoquardi* is mainly transmitted by *Hy. anatolicum*, although other possible vectors include *R. sanguineus* (Razmi et al. 2003). *Theileria lestoquardi* infections of small ruminants have been reported in Sudan, Egypt, India, Turkey, Iran, Saudi Arabia, and Oman (El Imam and Taha 2015).

Theileria luwenshuni and *T. uilenbergi* were described in China as pathogenic *Theileria* spp. transmitted by *Haemaphysalis qinghaiensis* ticks and initially referred to as *Theileria* sp. 1 (China) and *Theileria* sp. 2 (China), respectively. These parasites were initially confused with *T. ovis* and *T. lestoquardi*, but phylogenetic analysis of their 18S rRNA gene sequences showed that *T. luwenshuni* and *T. uilenbergi* group differently from *T. ovis* and *T. lestoquardi* (Schnittger et al. 2000, 2003; Yin et al. 2007). Following their classification as separate species, there are no descriptions of the pathogenic effects caused by *T. luwenshuni* and *T. uilenbergi.* However, outbreaks of ovine theileriosis, with high morbidity and mortality in sheep and goats from different regions of China, have long been reported, and although molecular studies to confirm the etiological agent are not available, they are likely associated with infections with these parasites. Reported clinical signs include fever, inappetence, and

cessation of rumination, rapid heartbeat dyspnea, weakness, listlessness, and swelling of superficial lymph nodes. Lethality rates were especially high among lambs and animals of 1–2 years of age (Luo and Yin 1997; Guo et al. 2002). Importantly, outbreaks of ovine theileriosis due to *T. luwenshuni* infection have been recently demonstrated by molecular detection from India and the UK (Phipps et al. 2016; Dhaygude et al. 2021). Furthermore, it has been reported that *T. luwenshuni* and *T. uilenbergi* were not able to transform lymphocytes (Luo and Yin 1997; Yin et al. 2007). Recently, *Theileria* sp. OT1 has been isolated from sheep in Spain and phylogenetic inference based on the 18S rRNA gene demonstrates that it is identical with *T. luwenshuni* suggesting that the distribution of this parasite is wider than has been previously assumed (Fig. 4, Nagore et al. 2004a, b). *Theileria uilenbergi* has also been identified in sika deer (*Cervus nippon*) and *T. luwenshuni* in Japanese serow (*Capricornis crispus*), suggesting that these may be their respective natural hosts (Fig. 4, Schnittger et al. 2003; Li et al. 2014; Liu et al. 2016).

Theileria separata, initially named *Haematoxenus separatus*, causes subclinical infections in sheep in Tanzania and Kenya, and is transmitted by *R. evertsi* (Uilenberg and Andreasen 1974; Uilenberg and Schreuder 1976; Young and Mchinja 1977). Recently, the 18S rRNA gene sequence of a *Theileria* sp. isolated from an infected sheep in China showed high similarity to the 18S rRNA gene sequence of a *T. separata* isolate from South Africa (Sun et al. 2019). This finding could indicate that either the geographical distribution of *T. separata* extends beyond sub-Saharan Africa, or that this represents a rare case, perhaps connected to sheep trade between South Africa and China. If the first scenario holds true, then a suitable vector needs to be identified, since *R. evertsi* is an afrotropical tick, not present in China (Walker et al. 2000).

Theileria ovis causes asymptomatic infections and seems to be widespread since it has been identified in Spain, France, Turkey, Sudan, and China. Recently, a *Theileria* sp. has been identified in sheep and goats in Malawi, Africa, which seems to be most closely related to *T. ovis* (Chatanga et al. 2021). However, this isolate, designated as *Theileria* sp. Malawi, represents a novel species as demonstrated in the inferred phylogenetic tree of 18S rRNA genes (Fig. 4, Table 2).

Two additional non-pathogenic isolates, *Theileria* sp. OT3 and *Theileria* sp. MK, have been reported in sheep of Spain and Turkey, and based on 18S rRNA sequences analysis, both represent novel species that await further characterization (Nagore et al. 2004a, b; Altay et al. 2007). Interestingly, *Theileria* sp. OT3 has been identified in several wild hosts such as red and roe deer (*Capreolus capreolus*) and in Pyrenean chamois (*Rupicapra pyrenaica*), suggesting a low host specificity. *Theileria recondita* is mentioned in some publications, but there is not enough information about this species and no molecular data are available. Thus, the existence of this parasite and demonstration that it represents a separate species would need confirmation (Alani and Herbert 1988; Schnittger et al. 2003).

Piroplasmids in companion animals

Equine piroplasmids

Theileria equi, the recently described *Theileria haneyi*, and *Babesia caballi* are obligate intraerythrocytic apicomplexan parasites that infect domestic equids (horse, donkey, and mule) as well as zebra (*Equus zebra*, *Equus quagga*, and *Equus grevyi*) and wild asses (*Equus africanus*, *Equus hemonius*, and *Equus kiang*) (Nuttall and Strickland 1910; Mehlhorn and

Schein 1998; Knowles et al. 2018; Librado and Orlando 2021). *Theileria equi* and *T. haneyi* are placed into a single monophyletic Clade IV (Equus group, Figs. 2 and 5) that is clearly distinguishable from *Theileria* s.s. (Clade V, Figs. 2 and 4) as inferred by phylogenetic analysis using single marker genes or phylogenomics (Schnittger et al. 2012; Jalovecka et al. 2019; Muñoz-Gomez et al. 2019). In contrast, *B. caballi* is the only piroplasmid species infecting horses that is placed into *Babesia* s.s. (Clade VI, Figs. 2 and 3). Infection by these hemoparasites causes equine piroplasmosis (EP), a globally significant disease, which is maintained in equid populations in areas where competent tick vectors are present (Schein 1988).

Fig. 5. Molecular phylogenetic tree of 18S rRNA gene sequences of *Theileria* (Clade IV, Equus group) infecting domestic horses. After alignment of 38 nucleotide sequences using MUSCLE, the Hasegawa-Kishino-Yano ($G + I$) parameter model was applied with a rate difference of 5 categories ($G = 0.11$, $I = 72.69\%$) to infer a maximum likelihood tree (Hasegawa et al. 1985). For tree estimation, 1388 positions were used. The 18S rRNA gene sequence of *Cytauxzoon felis* (AF39990) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

EP can have acute, peracute, and chronic manifestations. Clinical signs of acute and peracute cases are often non-specific and include fever, inappetence, malaise, tachycardia, and increased respiration rate, constipation followed by diarrhea, splenomegaly, anemia, and hemoglobinuria. Complications such as pneumonia and enteritis as well as abortion, due to pyrexia of the mare or intrauterine infection of the fetus, can occur. In the case of *B. caballi* infections, central nervous system involvement has been reported. Chronic infections are characterized by mild inappetence, loss of weight, and poor performance (Ueti et al. 2005; Laus et al. 2015; Ueti and Knowles 2018). Significant economic losses to the equine industry connected to EP are due to loss of activity, treatment, abortions, and death. Additionally, EP imposes restrictions on the international movement of horses (Rothschild 2013).

In addition to 33 ixodid tick species belonging to six genera (*Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus*) that have been identified and/or are suspected as vectors of EP, iatrogenic transmissions have been reported for *T. equi* and *B. caballi*, whereas transplacental transmission has been reported exclusively for *T. equi* (Scoles and Ueti 2015; Short et al. 2012; Wise et al. 2013; Sant et al. 2016, 2019). EP is endemic in many parts of the world, including Southern Europe, Asia, Central and South America, and Africa (Onyiche et al. 2019). Non-endemic countries include the United States (USA), Canada, the United Kingdom (UK), New Zealand, Japan, and Australia (Tirosh-Levy et al. 2020). However, outbreaks of *T. equi* have been reported in the UK and USA, endangering their current piroplasmosis-free status (Scoles et al. 2011; Short et al. 2012; Coultous et al. 2019).

The description of EP dates to the early twentieth century when the disease was initially described as either anthrax fever, biliary fever, a bilious form of African horse sickness, or equine malaria (Hutcheon 1890; Nunn 1894; Theiler 1901; Henning 1956). In 1901, one EP etiological agent was classified in the genus *Piroplasma* following the recognition of an intraerythrocytic protozoan parasite in the blood of horses from South Africa (Laveran 1901). At around the same time, the disease was reported in a group of imported mules in French Indo-China and, in 1908, in Sardinia, in a group of horses imported from Hungary (Bimbi 1916; Schein 1917).

Since its initial discovery, the taxonomy of *Piroplasma equi,* later reclassified as *Babesia equi* and then as *Theileri equi*, has changed several times (Laveran 1901; Levine et al. 1980; Schein et al. 1981). *Piroplasma caballi* (later reclassified as *Babesia caballi*) was discovered shortly after, in 1904, as the largest of two morphologically distinct parasites infecting horses in Zimbabwe (Nuttall and Stickland 1912). Although both parasites belong to the phylum Apicomplexa and are classified in the family Piroplasmida, only *B. caballi* is regarded as a true *Babesia* species (*Babesia* s.s.). In contrast, the discovery of schizogony in the lymphocytes of horses led to the reclassification of *B. equi* into *Theileria equi* as it was assumed at this time that this characteristic was unique to the true *Theileria* (*Theileria* s.s.) (Mehlhorn and Schein 1998). Many years later, phylogenetic analysis demonstrated that *T. equi* does not belong to *Theileria* s.s., but represents a distinct independent monophyletic lineage (Clade IV, Schnittger et al. 2012) that has been recently referred to as the Equus group (Jalovecka et al. 2019; Bhoora et al. 2020). In accordance with their taxonomic positioning, *B. caballi* and *T. equi* are transmitted transovarially and transstadially in the vector tick, respectively (Ueti and Knowles 2018).

Piroplasmids of the Equus group split into at least five 18S rRNA gene sublineages that are commonly referred to as genotypes A, B, C, D, and E. Reference to isolates belonging to

these sublineages as genotypes, combined with the fact that coinfection is frequently observed, may have obscured the view that they most likely represent a complex of at least five different species, as strongly suggested by phylogenetic analysis using the 18S rRNA gene (Table 3, Fig. 5). The term *genotype* refers to allelic variants between which genetic exchange takes place (a characteristic that is sufficient, though not necessary, for species definition), which in turn implies that isolates represent variants of a single species. It would therefore be preferable to refer to them in an impartial manner, such as sublineages or isolates of types A–E, until evidence has been presented that they correspond to either a single species or a complex of different species infecting the same host. Noteworthy, delineation of species using the 18S rRNA gene has been very widely used and is a highly reliable taxonomic and phylogenetic tool for classifying *Babesia*, *Theileria*, and other hemoprotozoan species (Schnittger et al. 2012; Greay et al. 2018). Molecular phylogeny based on the 18S rRNA gene reveals that piroplasmids infecting equids diverge into two major lineages, one composed of sublineages A (found in most countries and all continents), B (endemic in Africa and the Mediterranean region), and E (present in Northern and Eastern Europe, and Middle and the Far East), whereas the other lineage diverges into the sublineages D (endemic in Africa, the Mediterranean region, and Middle East) and C (distributed in all continents). Importantly, A and C are the genetically most distant types corroborating that they represent two independent species, albeit displaying a common, widely overlapping global distribution. It would appear that these two specific types may have been distributed by the introduction and worldwide trading of the domestic horse. Groups E and B, which exhibit a closely related genetic identity, show a non-overlapping geographic distribution that supports the hypothesis that they represent distinct species (Tirosh-Levy et al. 2020; Bishop et al. 2020).

Consistent with this view, Dahmana et al. (2019) reported two putative novel species, on one hand, *Theileria* sp. (Africa), which corresponds to types C and D, and, on the other hand, *Theileria* sp. (Europa), which corresponds to type E. Consequentially, a recent study including field horses, race horses, and wild zebras showed that infections with types A, B, C, and D represent independent stochastic events, strongly supporting that each type corresponds to an independent species (Bhoora et al. 2020). Interestingly, type B was confined to zebras, suggesting that this type is rather associated with wild equids and, perhaps, other wild species.

When types A to E are finally recognized as different species, it will be important to determine which represents *T. equi* taxonomically. Once a type has been accepted as *T. equi* (*T. equi* s.s.), then consequently isolates of all other sublineages will need to be referred to as *T. equi*-like (*T. equi* s.l.) or given exclusive isolate designations. In the context of this study, we have sequenced the 18S rRNA and *cox1* gene of the reference isolate on which the redescription of *T. equi* is based and determined it as type A (GenBank: accession no. OL638195 and OL672235; Table 3, Fig. 5, Mehlhorn and Schein 1998). This finding agrees with the type of the *T. equi* USDA (United States Department of Agriculture) reference strain, and therefore, it seems to be most appropriate to refer to type A as *T. equi* (or *T. equi* s.s.). Accordingly, naming or referring to other type isolates as *T. equi* should be discouraged; however, they might be referred to as *T. equi*-like (or *T. equi* s.l.). Phylogenetic analysis based on the 18S rRNA gene and the acquisition of the first genome sequence of the USDA Florida strain of *T. equi* (type A) has provided insight into the phylogenetic placement and taxonomic classification of this parasite and these data suggest that creation of a new genus, here referred to as Equus group, might be appropriate (Kappmeyer et al. 2012; Schnittger et al. 2012; Jalovecka et al. 2019).

Table 3 Piroplasmid species in equids

[a]Species	Type (= genotype), proposed species	[e]Geographic distribution	Pathogenicity and remarks	Candidate tick vectors	Clade	Reference
[d]*T. equi*	Type A	Most countries and all continents except Australia	Pathogenic, genome available	*Amblyomma* *Rhipicephalus* - *R. bursa* - *R. sanguineus* s.l. *Dermacentor* - *D. reticulatus* - *D. marginatus* *Hyalomma* - *H. marginatum* *Haemaphysalis* - *H. punctata* *Ixodes* - *Ixodes ricinus*	Clade IV (Equus group)	Bhoora et al. 2009, 2010; Kappmeyer et al. 2012
[b]*Theileria haneyi*	Type C	North America, South Africa	Moderate pathogenic, genome available			Knowles et al. 2018; Bhoora et al. 2020
[b]*Theileria equi*-like	Type C [c]*Theileria* sp. (Africa)	All continents except Australia	Non-pathogenic			Bhoora et al. 2009, 2010
	Type D, [c]*Theileria* sp. (Africa)	Africa, Mediterranean region, Middle East	Non-pathogenic; frequently detected in zebras and donkey			Salim et al. 2010; Qablan et al. 2012, 2013; Bhoora et al. 2020; Coultous et al. 2019
	Type E, [d]*Theileria* sp. (Europa)	Northern and Eastern Europe, Middle and Far East	Non-pathogenic			Salim et al. 2010; Qablan et al. 2012, 2013; Wang et al. 2019
	Type B	Africa and Mediterranean region	Non-pathogenic; frequent in zebra, infrequent in horses			Bhoora et al. 2009, 2010; Bhoora et al. 2020
Babesia caballi	Types A, B (B1), and C (B2)	All continents except Australia	Moderately pathogenic		Clade VI (*Babesia* s.s.)	Nuttall and Stickland 1912

[a]Since *T. equi* type A represents the reference species *T. equi* s.s., types B–E are referred to as *Theileria equi*-like or *T. equi* s.l.

[b]Linkage of *Theileria haneyi* with genotype C is observed suggesting both represent a single species (Bhoora et al. 2020)

[c]Genotypes C and D have been reported to represent the putative novel species *Theileria* sp. Africa

[d]Type E has been reported as putative novel species *Theileria* sp. Europa (Dahmana et al. 2019)

[e]The geographic distribution is given according to Tirosh-Levy et al. (2020)

The recently described *T. haneyi* has been isolated and sequenced from a stray horse captured at the USA-Mexico border near Eagles Pass, Texas (Knowles et al. 2018). Furthermore, it has been clearly distinguished from *T. equi* type A by phylogenomics and absence of *ema-1* compared to *T. equi* type A (USDA reference strain), demonstrating that the two 18S rRNA sublineages A and C represent two distinct piroplasmid species (Knowles et al. 2018). The presence of *T. haneyi* has since been reported to occur in several countries from North and South America, Africa, and Asia (Knowles et al. 2018; Bhoora et al. 2020; Bishop et al. 2020; Mshelia et al. 2020).

Phylogenetically, as demonstrated by 18S rRNA gene comparison, *T. haneyi* segregates with *T. equi*-like type C sequences from horses and zebra (Fig. 5) (Knowles et al. 2018; Bishop et al. 2020; Manna et al. 2018). Furthermore, *T. haneyi* infections were reported to occur in South African equids infected with *T. equi*-like type C and results strongly suggested that there is an association between *T. haneyi*, and *T. equi ema-1*, and 18S rRNA type C (Bhoora et al. 2020). The phylogenetic clustering of *T. haneyi* with *T. equi*-like type C therefore suggests that these parasites may represent one species and further implies that other *T. equi* types (B, D, E) also represent distinct parasite species. Based on these observations and considerations, delineation of *T. haneyi* with remaining type C isolates warrants further investigation. To this aim, analysis of the variable *cox1*, *cox2*, or *cox3* gene sequence may facilitate distinguishing and delineating piroplasmid species of the Equus group, as has been successfully achieved for *Babesia* and *Cytauxzoon* species of other piroplasmid lineages (Sivakuma et al. 2019; Hrazdilová et al. 2020; Panait et al. 2021).

Experimental infections with *T. haneyi* have been shown to induce minimal clinical disease in spleen-intact horses, characterized by mild changes in packed cell volume (PCV) and occasional development of fever during the acute phase (Sears et al. 2019). In contrast, *T. equi* type A, which has been identified in both endemic and non-endemic countries, has been reported to cause infections of varying degrees, inducing severe anemia, sometimes resulting in mortality in domestic equids (Hall et al. 2013; Manna et al. 2018; Bishop et al. 2020; Sebastian et al. 2021). Furthermore, type A has been linked to two EP outbreaks in the USA and was found to be associated with clinical cases of EP in Italy and Israel while types B, C, and D appear to be non-pathogenic (Hall et al. 2013; Manna et al. 2018; Tirosh-Levy et al. 2020).

Generally, the reported prevalence of *B. caballi* globally is much lower than that reported for *T. equi*. However, in Turkey and some Asian countries, the opposite scenario appears to be the rule (Acici et al. 2008; Sloboda et al. 2011; Munkhjargal et al. 2013; Tirosh-Levy et al. 2020). *Babesia caballi* infections are self-limiting, usually persisting for 1–4 years without any clinical signs until they are naturally eliminated. Furthermore, sequestration of piroplasms to the bone marrow generally results in extremely low parasitemia levels, rarely exceeding 1% in naturally infected *B. caballi* horses (Schein 1988; de Waal 1992; Sant et al. 2016). Genetic variants based on the 18S rRNA gene of *B. caballi* and classified as types A, B (or B1), and C (or B2) have been identified in Asia, Europe, Africa, and South America (Fig. 3; Aziz and Al-Barwary 2020; Bhoora et al. 2009; Braga et al. 2017; Manna et al. 2018; Munkhjargal et al. 2013; Ros-García et al. 2013; Tirosh-Levy et al. 2020).

Several drugs have been described for alleviating EP clinical signs, among which imidocarb propionate is the most efficient (Grause et al. 2013; Ueti and Knowles 2018; Onyiche et al. 2019). However, it was recently shown that treatment with this drug was not effective against *T. haneyi* and its effect against *T. equi* was diminished in *T. haneyi*-*T. equi* coinfection cases.

Thus, studies focusing on alternative chemotherapeutic agents are required (Sears et al. 2020). In addition to chemotherapy, supportive care, including intravenous fluids, anti-inflammatory drugs, pain management, and blood transfusion, are often needed (Wise et al. 2013).

In tropical and subtropical regions, where tick infestation of equids is a big challenge, acaricides such as organophosphates, pyrethroids, and amidines are used to alleviate tick burdens and partially prevent EP transmission (Ueti and Knowles 2018). Prophylactic treatment of horses is also sometimes applied. Additionally, special care is recommended to avoid transmission through blood-contaminated equipment or blood transfusions (Onyiche et al. 2019). In EP non-endemic countries, prevention is carried out by imposing strict regulations for the import of horses from endemic regions, which include serological testing, acaricide treatment, chemotherapy, and quarantine (Wise et al. 2013). Notably, as few as one *T. equi*-infected tick is sufficient to transmit the parasite to a naïve horse (Ueti et al. 2005). This illustrates the high risk of introducing chronically infected horses into a non-endemic area where competent vectors occur, especially considering the likelihood of acaricide resistance development (George et al. 2004). Vaccination is considered a potentially useful preventive tool; however, no commercial vaccines are presently available for EP (OIE 2021).

Babesia in dogs

Piroplasmids infecting dogs all belong to the genus *Babesia*, where they group within the *Babesia* s.s. clade (Clade VI, Figs. 2 and 3) and into two distinctly different *Babesia* s.l. clades, referred to as the Western clade (Clade II, Figs. 2 and 6) and the *Babesia vulpes* group (Clade Ib, Figs. 2 and 7) (Jalovecka et al. 2019).

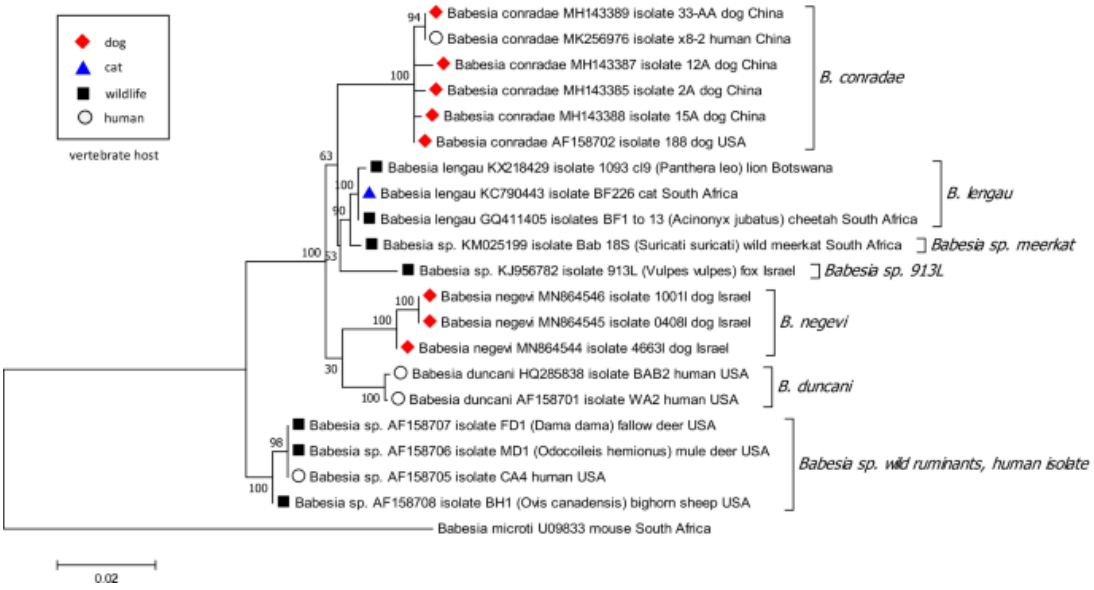

Fig. 6. Molecular phylogenetic tree of 18S rRNA gene sequences of *Babesia* s.l. (Clade II, Western group). After alignment of 21 nucleotide sequences using MUSCLE, the Tamura-Nei ($G + I$) parameter model was applied with a rate difference of 5 categories ($G = 0.12$, $I = 75.37\%$) to infer a maximum likelihood tree (Tamura and Nei 1993). For tree estimation, 1364 positions were used. The 18S rRNA gene sequence of *Babesia microti* (U09833) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

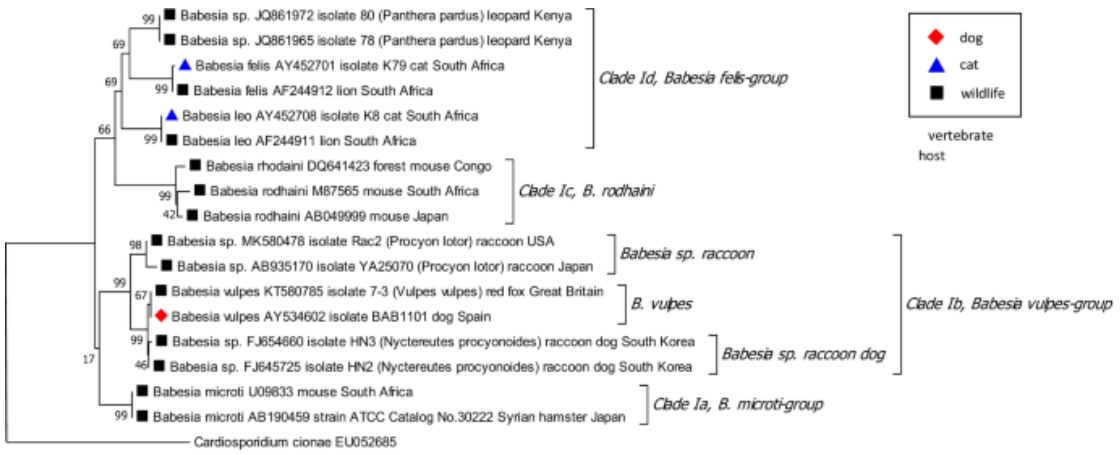

Fig. 7. Molecular phylogenetic tree of 18S rRNA gene sequences of *Babesia* s.l. (Clade I, *B. microti* group). After alignment of 18 nucleotide sequences using MUSCLE, the Tamura-Nei ($G = 0.15$) parameter model was applied to infer a neighbor-joining tree (Saitou and Nei 1987; Tamura and Nei 1993). The differences in sequence composition were considered in evolutionary comparisons (Tamura and Kumar 2002). For tree estimation, 1506 positions were used. The 18S rRNA gene sequence of *Cardiosporidium cionae* EU052685 was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

Well-described *Babesia* spp. infecting domestic dogs include *B. vogeli*, *B. canis*, *B. rossi*, *B. gibsoni*, *Rangelia vitalii*, *B. vulpes*, *B. conradae*, *B. negevi*, and *Babesia* sp. Coco (Table 4). Due to some peculiarities of its life cycle, such as the invasion of and propagation in leukocytes and endothelial cells, and the distinct clinical signs observed after *R. vitalii*-infection, the parasite was proposed to belong to a separate genus and given the generic name *Rangelia* (Carini and Maciel 1914; Loretti and Barros 2005; Soares et al. 2011). However, our phylogenetic analysis of its 18S rRNA gene sequence clearly places this species within the *Babesia* s.s. Clade VI (see Fig. 3), confirming previous phylogenetic analysis based on 18S RNA and hsp70 genes by Soares et al. (2011). It is most reasonable to assume that invasion and propagation of the parasite in leukocytes and endothelial cells represents an autapomorphy within *Babesia* s.s. This is reinforced by the observation that *R. vitalii* places as a sister taxon to a subclade of *Babesia* s.s. parasites (see carnivore clade D, Fig. 3; Soares et al. 2011; Inácio et al. 2019). Furthermore, the traditional definition of *Babesia* s.s. as piroplasmids that exclusively infect erythrocytes is based on a symplesiomorphy as it is also presented by *Babesia* s.l. parasites (Clades I and II). Importantly, it is well established that symplesiomorphies are not suited to define a taxonomic group. Therefore, the taxonomic classification of this parasite needs revision, to correspond with its phylogenetic placement into *Babesia* s.s. (Clade VI). Interestingly, a parallel situation with respect to *R. vitalii* is observed for transforming *Theileria* parasites (e.g., *T. parva*, *T. annulata*, *T. lestoquardi*, *T. taurotragi*) displaying the autoapomorphy of leukocyte transformation, resulting in unique and severe cancer-like pathologies after infection; however, as inferred by molecular phylogeny, they clearly place with non-transforming *Theileria* into *Theileria* s.s (Clade V).

Table 4 *Babesia* infecting domestic dogs

Species	Candidate or confirmed tick hosts	Distribution	Pathogenicity	Merozoite size[b]	Clade[c]	Reference
B. vogeli	[a]*Rhipicephalus sanguineus* s.l.	Worldwide	Subclinical or mild in adults; severe in pups	L	*Babesia* s.s. (Clade VI)	Uilenberg et al. 1989; Zahler et al. 1998; Carret et al. 1999; Schnittger et al. 2012
B. canis	[a]*Dermacentor reticulatus*	Europe	Mild to severe depending on the individual	L		
B. rossi	[a]*Haemaphysalis elliptica*, *Haemaphysalis leachi*	Southern Africa, Nigeria, Sudan	Severe	L		
R. vitalii	[a]*Amblyomma aureolatum*	South America	Severe	S/M		Loretti and Barros 2005; Soares et al. 2011
Babesia sp. Coco	nk	USA	Severe in immunosuppressed dogs	L		Birkenheuer et al. 2004; Sikorski et al. 2010
B. gibsoni	[a]*H. longicornis*, *H. bispinosa*, *R. sanguineus* s.l.	Southeast Asia, USA, Australia, Europe	Mild to severe depending on the individual	S		Patton 1910
Babesia sp. Akita610	*Ixodes ovata*	Japan	Asymptomatic	nk		Inokuma et al. 2003
B. negevi	*Ornithodoros tholozani*	Israel	Severe	S/M	Western group (Clade II), *Babesia* s.l.	Baneth et al. 2020
B. conradae	nk	USA, China	Severe	S		Kjemtrup et al. 2006
B. vulpes	*D. reticulatus*, *Ixodes hexagonus*, *I. ricinus*, *I. canisuga*, *R. sanguineus* s.l.	Europe, North America	Severe	S	*Babesia vulpes*-group (Clade Ib), *Babesia* s.l.	Baneth et al. 2015, 2019

nk, not known

[a]These tick species have been experimentally confirmed as vector

[b]L = large: 4.5 to 6 µm long; M = medium: 1.2 to 4.8 µm long; S = small: 2.5 to 3 µm long

[c]Clades according to Schnittger et al. (2012)

The most recent addition to the array of canine *Babesia* spp. is *Babesia negevi*, a parasite detected in dogs of Israel, which belongs with *B. conradae* into Clade II (Western group, Fig. 6) (Kjemtrup et al. 2006; Baneth et al. 2020). Conversely, *B. vulpes* places into Clade Ib (*Babesia vulpes* group, Fig. 7) and together with *B. negevi* and *B. conradae*, is considered a *Babesia* s.l. parasite. All remaining canine-infecting species belong to the *Babesia* s.s. group (Clade VI, Fig. 3) (Schnittger et al. 2012; Baneth et al. 2015; Baneth 2018a).

As mentioned above, no canine piroplasmid belongs to the *Theileria* and *Cytauxzoon* genera; however, *B. vulpes* had originally been described as a *Theileria* s.s. (*T. annae*) but was later reclassified in *Babesia* s.l. of Clade Ib (Baneth et al. 2015, 2019; Jalovecka et al. 2019). Nonetheless, incidental infections of dogs with ruminant *Theilera* spp. have recently been detected by molecular methods in dogs from Myanmar, though it is considered unlikely that these parasites prosper in canids as only hosts (Bawm et al. 2021). In addition, *T. equi* has been identified in dogs; however, it is yet unclear whether this species can propagate and be further transmitted in this host species (Beck et al. 2009; Rosa et al. 2014; Inácio et al. 2019; Bishop et al. 2020). Furthermore, a *Babesia* isolate has been identified in a dog in Nigeria (GenBank: accession no. AB935162, without published report), which was found to be most closely related to *B. hongkongensis* infecting cats. In addition, the isolate *Babesia* sp. Akita has been identified from ticks sampled from a dog in Japan. It was found to be most closely related to two *Babesia* sp. isolates, one named Iwate248 from a Japanese black bear, Japan, and another from a captive maned wolf in the USA (Fig. 3, Inokuma et al. 2003). However, the validity of these isolates as species would need to be confirmed in future studies.

Noteworthy, an accidental infection of dogs with *B. caballi* has also been recently reported (de Sousa et al. 2018).

Infection of dogs by *Babesia* spp. results in very varying clinical presentations, depending on the species as well as the age, immune status, and concomitant infections of the affected animal. Clinical signs can include fever, anemia, lethargy, anorexia, thrombocytopenia, tissue anoxia, and organ dysfunction, among others, and infections can be fatal (Baneth 2018b).

For some species, such as *B. vogeli*, at least one tick species has been identified as vector of transmission, while for others, this information is either not available, as in the case of *B. conradae*, or is only speculative, as in the case of *B. negevi*. Interestingly, *B. negevi* parasite DNA was detected by PCR in the soft tick *Ornithodoros tholozani*, and if confirmed, this would be the first canine *Babesia* described to be transmitted by an argasid tick (Table 4, Baneth et al. 2020).

The geographical distribution of canine *Babesia* spp. according to Baneth (2018a) and Baneth et al. (2020) is shown in Table 4. This distribution could partly be confirmed by a molecular survey focused on the detection of the five canine species *B. canis, B. vogeli, B. gibsoni*, *B. rossi*, and *B. conradae* in 100,000 dog samples sent to a commercial diagnostic laboratory from 52 countries of all continents, excluding Africa. *Babesia canis*, *B. vogeli*, and *B. gibsoni* were the most prevalent species found in Europe, South America, and Asia, respectively. Interestingly, *B. gibsoni* was the most prevalent canine *Babesia* sp. among dog samples from North America, and this was, overall, the most frequent species detected among the studied samples from all regions (Birkenheuer et al. 2020). *Babesia gibsoni* was originally described in Asia and could have increased its distribution range through the expansion of its tick vector, *Haemaphysalis longicornis*, which was recently found in North America (Saleh et al. 2021). Transmission by additional tick vectors, which include the widespread *R. sanguineus* s.l., may have contributed to the expansion of *B. gibsoni* outside its original geographical range, although this has not yet been confirmed (Baneth 2018a, b). In addition to transmission through tick vectors, transmissions by blood transfusion and bites among fighting dogs, as well as transplacental transmission, have been reported for *B. gibsoni* (Fukumoto et al. 2005; Baneth 2018a). The latter could constitute a highly efficient way for the transboundary expansion of this parasite independent of the tick vector especially in the case of pure breed dogs destined to be exported.

For two canine *Babesia* spp., it has been possible to determine the wild canid that constitutes their natural hosts. This is the case for the black-backed jackal (*Canis mesomelas*) for *B. rossi* and the fox (*Vulpes vulpes*) for *B. vulpes*. In both cases, parasite prevalence in the natural host populations is quite high and infections are subclinical, as expected for long-lasting, well-adapted host-parasite interactions (Baneth et al. 2015; Penzhorn et al. 2017; Shabangu et al. 2021). In addition, *R. vitalii* infections of free-ranging neotropical wild canids including the maned wolf (*Chrysocyon brachyurus*), the crab-eating fox (*Cerdocyon thous*), and the pampas fox (*Lycalopex gymnocercus*) have been reported and these hosts may represent the natural reservoir of this species (Soares et al. 2014; Fredo et al. 2015; Silveira et al. 2016; de Lorenzo et al. 2021).

It is possible that domestic dogs first acquired these infections through the bite of compatible ticks when accompanying humans in early settlements. Although reports of babesiosis of dogs are only available from the late nineteenth century, first exposure of domestic dogs to *B. rossi* likely took place quite recently at the seventeenth century when European colonizers

settled in the southern region of South Africa. Thus, local dog-parasite interactions are relatively recent and this could explain why babesiosis by *B. rossi* is highly pathogenic in domestic dogs. Following the same reasoning, the association between *B. vogeli* and the domestic dogs might be the oldest since dog infections by this parasite generally result in subclinical or mild disease (Penzhorn 2011).

Canine babesiosis has attracted considerable research efforts due to the social impact of companion dog diseases and the resulting interest of pharmaceutical companies. Several drugs and drug combinations have been described for the treatment of acute canine babesiosis; however, they are not able to eliminate the parasite leading to asymptomatic carrier animals that may relapse and transmit the infection. Importantly, effective chemotherapy depends on the infecting agent, since drug susceptibility varies between large and small *Babesia* spp. Therefore, a correct diagnosis before treatment is of paramount importance (Baneth 2018b). Tick control with systemic or cutaneously administered synthetic acaricides decreases the risk of transmission of *Babesia* as well as other tick-borne pathogens (Pfister and Armstrong 2016). Effective commercial vaccines against *B. canis* and *B. rossi* based on culture supernatant antigens have been developed (Schetters 2005; Schetters et al. 2007; Freyburger et al. 2011). In addition, an experimental vaccine against *B. canis* based on a recombinant form of an immunodominant antigen present in culture supernatants proved protective against challenge, suggesting that this type of approach may also be effective against other *Babesia* species (Moubri et al. 2018). Finally, canine blood donors should be regularly screened to prevent transmission of *Babesia* sp. parasites through blood transfusion (Wardrop et al. 2016).

Babesia and *Cytauxzoon* in cats

Cats can be infected by two genera of piroplasmids, *Babesia* and *Cytauxzoon* (Fig. 2, Schnittger et al. 2012). While felid-infecting *Babesia* are placed within the *Babesia* s.s. clade (Clade VI, Fig. 3), the Western group (Clade II, Fig. 6), and the *B. felis* group (Clade Id, Fig. 7), all *Cytauxzoon* species segregate into a single Clade III (Fig. 8).

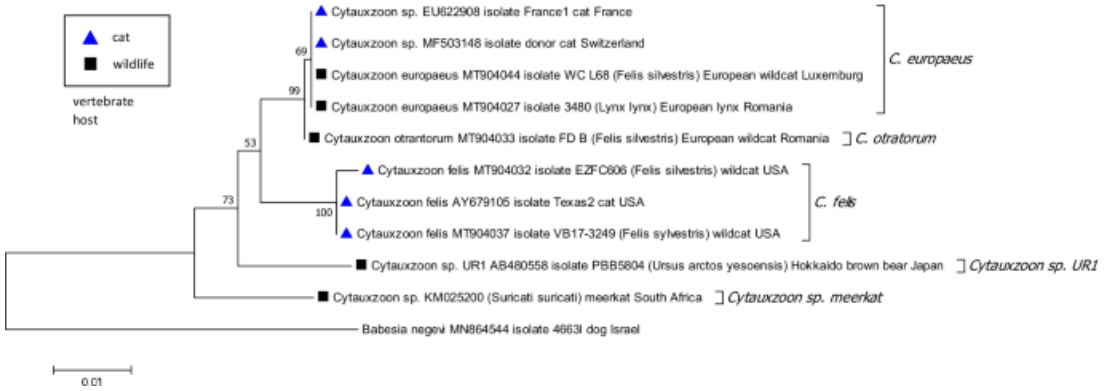

Fig. 8. Molecular phylogenetic tree of 18S rRNA gene sequences of *Cytauxzoon* (Clade III). After alignment of 11 nucleotide sequences using MUSCLE, the Tamura-Nei ($G + I$) parameter model was applied with a rate difference of 5 categories ($G = 0.10$, $I = 45.87\%$) to infer a maximum likelihood tree (Tamura and Nei 1993). For tree estimation, 1114 positions were used. The 18S rRNA gene sequence of *Babesia negevi* (MN864544) was used as an outgroup. Bootstrap values were estimated based on 1000 replicates. The length of the bar corresponds with the indicated number of substitutions per site. Phylogenetic analysis was carried out using MEGA7 (Kumar et al. 2016)

Reports on feline babesiosis are scarce especially when compared to the vast number of publications on babesiosis of dogs and other domestic species, as these infections are usually asymptomatic except for some species found in southern Africa. Several *Babesia* sp. infecting domestic cats have been identified using molecular methods. Those associated with wild and domestic felines include *B. panickeri*, *B. hongkongensis*, *B. canis* subspecies *presentii*, and a recently detected *Babesia* sp. from South Africa, provisionally named *Babesia* sp. Western Cape and groups into *Babesia* s.s. (Clade VI, Fig. 3), whereas *B. lengau* groups into the Western clade (Clade II, Fig. 6), and *B. felis* and *B. leo* into the *B. microti* group (Clade I, Fig. 7); the latter two clades represent *Babesia* s.l. (Table 5, Penzhorn et al. 2001; Baneth et al. 2004; Bosman et al. 2007, 2010, 2019; Schnittger et al. 2012; Wong et al. 2012; Jalovecka et al. 2019; Panicker et al. 2020; Penzhorn and Oosthuizen 2020). Additionally, *Babesia* spp. that are typically found in other hosts, such as *B. canis*, *B. vogeli*, *B. gibsoni*, and *B. vulpes* from canines, *B. microti* from rodents, or *B. lohae* from the marsupial brushtail possum (*Trichosurus vulpecula*), have been detected in domestic cats using molecular methods. However, most of them seem accidental infections, as they represent rare incidental findings. On the other hand, *B. microti* and *B. vogeli* have been reported to infect cats with moderate prevalence, but cause asymptomatic infections (André et al. 2014, 2015; Malheiros et al. 2016; Baneth et al. 2015, 2019; Loh et al. 2018; Greay et al. 2018; Bosman et al. 2019; Penzhorn and Oosthuizen 2020). Noteworthy, 18S RNA gene sequences ($n = 2$, KP402163-4) most closely related to *T. cervi* (MW008528) and 18S RNA gene sequences ($n = 3$, KP410267-9) most closely related to *Theileria* sp. isolated from African buffalo (*Syncerus caffer*) (HQ895976) have been reported from domestic cats in Brazil. In addition, 18S RNA gene sequences of an isolate designated *Theileria* sp. Cat ($n = 5$, KP410270-3, KF970930), highly similar with sequences of a *T. equi*-like piroplasmid (MZ4910896, MZ491096) recently reported from tapir (*Tapirus terrestris*), have been identified in domestic cats in Brazil (André et al. 2014, 2015; Silva et al. 2021). As reported sequences are relatively short, they could not be integrated in our tree. However, the findings show that *Theileria* s.s.-related and *T. equi*-related piroplasmids are able to infect cats. It will be important to determine whether these piroplasmids can propagate in domestic cats and which is their natural host. In the case of *Theileria* sp. Cat, the finding of similar sequences in tapir suggests that they may represent spillover or accidental infections in domestic cats (André et al. 2014, 2015).

Table 5 *Babesia* spp. infecting domestic cats

Species	Wild host	Country	Clade	Reference
Babesia sp. Western Cape	nk	South Africa	*Babesia* s.s. (Clade VI)	Bosman et al. 2019
B. hongkongensis	nk	Hong Kong		Wong et al. 2012
B. canis presentii	nk	Israel		Baneth et al. 2004
Babesia panickeri	Asian lion (*Panthera leo persica*)	India		Panicker et al. 2020
B. lengau	Cheetah (*Acinonyx jubatus*), African lion (*Panthera leo*)	South Africa	Western group (Clade II)	Bosman et al. 2010, 2013
B. felis	African lion (*Panthera leo*)	South Africa	*Babesia felis* group (Clade Id)	Penzhorn et al. 2001
B. leo	African lion (*Panthera leo*)	South Africa		Penzhorn et al. 2001

nk, not known

The first clinical case of feline babesiosis was reported in South Africa, which remains the country where most clinical cases are observed. Clinical signs in domestic cats are associated with infections of *B. felis*, *B. leo*, *B. lengau*, *B. panickeri*, and *Babesia* sp. Western Cape and include lethargy, anorexia, anemia, and, in some cases, pyrexia. Information on ticks involved in the transmission of feline *Babesia* spp. is not yet available (Bosman et al. 2013, 2019; Panicker et al. 2020; Penzhorn and Oosthuizen 2020).

The genus *Cytauxzoon* is known primarily for the infection of wild and domestic felines (Feliformia suborder, Felidae family), but infections were also found in meerkats (Herpestidae family of the same suborder) and other carnivores that include the Hokkaido brown bear (*Ursus arctos yesoensis*) and the Japanese black bear (*Ursus thibetanus japonicus*, both from the Caniformia suborder, Ursidae family) (Jinnai et al. 2010; Sherrill and Cohn 2015; Leclaire et al. 2015; Alvarado-Rybak et al. 2016; Wang et al. 2017; Moustafa et al. 2020). Notably, this genus was initially defined based on a parasite infecting a small antelope in South Africa, which invaded erythrocytes and formed schizonts in histiocytes. This piroplasmid, referred to as *Cytauxzoon sylvicaprae*, had similar features to *T. parva* and was thus proposed to belong to the Theileridae family, but as it was not found in lymphocytes, a separate genus, *Cytauxzoon*, was created (Neitz and Thomas 1948). In the 1970s, domestic cats from Missouri, USA, were reported to be infected with a highly virulent *Cytauxzoon*-like pathogen which was later named *C. felis* (Wagner 1976; Kier et al. 1982). Experimental infection of cats with *C. felis* showed a high rate of mortality (Ferris 1979). The advent of molecular methods for species discrimination demonstrated that *Cytauxzoon* infections are exclusively associated with carnivores, and it can thus be concluded that the parasite referred to as *C. sylvicaprae* in the 1940s was likely a *Theileria* sp. Indeed, it was shown that *Theileria* parasites can invade different mononuclear cells, thus invalidating the classification of the parasite found by Neitz and Thomas (1948) as a separate genus from *Theileria* sp. based on the absence of lymphocyte invasion (Spooner et al. 1989).

The phylogenetic placement of the *Cytauxzoon* genus has been controversial. In the analysis of Schnittger et al. (2012) based on 18S rRNA gene sequences, *C. felis*, *C. manul*, and *Cytauxzoon* sp. UR1 isolated from a Hokkaido brown bear fell in a strongly supported monophyletic group (designated Clade IIIb), clearly separated from *Theileria* s.s. and more closely related to some *Theileria* s.l. species. On the other hand, a phylogenetic analysis using concatenated mitochondrial genes (*cox1*, *cox2*, and *cox3*) together with the 18S rRNA gene integrated *C. felis* and *Theileri*a s.s. into a single monophyletic group (Schreeg et al. 2016). Recent genomic evidence, however, further supports that *Cytauxzoon* sp. and *Theileria* s.s. parasites represent independent evolutionary lineages. First, analysis of piroplasmid cysteine C1A proteases (C1A-Cp) showed that *C. felis*-C1A-Cp sequences segregate with high support and consistently as a sister taxon to sequences of *Theileria* s.s. (Ascencio et al. 2018). Additionally, the pattern of *N*-glycosylation of *C. felis* and *Theileria* s.s. species, as inferred by the array of glycosyl transferases encoded in their genomes, is decisively different. While *C. felis* can produce an N-glycan chain composed of two *N*-acetyl-glucosamine (NAcGlc) and one mannose molecule, and subsequently transfer it to a nascent protein, *Theileria* s.s. parasites are limited to the generation of a dolichol-P-linked *N*-glycan, composed of two NAcGlc molecules, which are not transferred to proteins. In other words, *Cytauxzoon* sp. proteins display *N*-glycans, while *Theileria* s.s. proteins do not, which highlights possible important differences in the biology of these two groups of piroplasmids (Florin-Christensen et al. 2021). Furthermore, in two recent phylogenomic analyses, one based on 90 nucleus-encoded genes and another on 35 apicoplast-encoded genes, *C. felis* was placed consistently and with strong support as a sister taxon to *Theileria* s.s. (Clade V) and *T. equi* (Clade IV) (Muñoz-Gomez et al. 2019).

Cytauxzoon felis is currently considered an emerging pathogen of veterinary importance in the USA (Table 6). Since its first detection in Missouri, its geographical distribution has expanded to several south eastern and south-central states (Wang et al. 2017). *Cytauxzoon felis*-infected domestic cats develop fever, anorexia, dehydration, icterus, lethargy, and hepatosplenomegaly and may die in a few days (Sherrill and Cohn 2015). The natural host of

C. felis in the USA is thought to be the bobcat (*Lynx rufus*), in which infections are usually asymptomatic (Glenn et al. 1983). However, the parasite has also been detected causing asymptomatic infections in Florida panthers (*Puma concolor coryi*) and Texas cougars (*P. concolor stanleyana*), and in captive-born tigers kept in a *C. felis*-endemic region of the USA (Yabsley et al. 2006; Lewis et al. 2012). Molecular studies confirmed that the principal tick vector of *C. felis* is the lone star tick, *Amblyomma americanum*, whose distribution overlaps with that of the natural host, the bobcat. *Dermacentor variabilis*, the American dog tick, has been reported as a competent vector; however, this could not be confirmed in subsequent experiments (Blouin et al. 1984; Kocan et al. 1992; Reichard et al. 2009, 2010; Saleh et al. 2021).

Table 6 *Cytauxzoon* spp. infecting domestic cats

Species	Wild host	Country	Reference
C. felis	Bobcat (*Lynx rufus*); Florida panther (*Puma concolor coryi*); Texas cougar (*P. concolor stanleyana*)	USA	Kier 1979; Allsopp and Allsopp 2006; Yabsley et al. 2006
C. europaeus	Wildcat (*Felis silvestris*), Eurasian lynx (*Lynx lynx*)	Europe	Panait et al. 2021
C. banethi	Wildcat (*Felis silvestris*), Eurasian lynx (*Lynx lynx*)	Romania	Panait et al. 2021
C. otrantorum	Wildcat (*Felis silvestris*)	Romania	Panait et al. 2021
Cytauxzoon sp. Kozhikode	nk	India	Malangmei et al. 2021

nk, not known

Cytauxzoon sp. has also been detected in domestic cats of several European countries, including Spain, France, Portugal, Italy, Switzerland, and Germany, and was likely erroneously referred to as *C. felis*. The common clinical sign encountered has been a mild anemia and only in a very few exceptionally cases the infection was associated with severe clinical disease and mortality (Criado-Fornelio et al. 2004, 2009; Carli et al. 2012; Alho et al. 2016; Wang et al. 2017; Nentwig et al. 2018; Panait et al. 2020). Importantly, a recent study has shown that the 18S rRNA gene of the causative agent of cytauxzoonosis in a cat from Germany displayed the highest identity with those of other European *Cytauxzoon* spp., but it was distantly related to sequences of *C. felis* from the USA (Panait et al. 2020). Thus, *Cytauxzoon* spp. infecting cats in Europe belong to any of the recently described species, *C. europaeus*, *C. otrantorum*, or *C. banethi*, that have been identified in their natural hosts, the European wildcat (*Felis silvestris*) and the European lynx (*Lynx lynx*) (Panait et al. 2021). Interestingly, phylogenetic analysis using the 18S rRNA gene segments of 1000 to 1200 bp length of *Cytauxzoon* isolates from European wildcats and the Eurasian lynxes did not discriminate between the three European *Cytauxzoon* spp. isolates. Nonetheless, when *cox1* and *cox2* nucleotide sequences of the *Cytauxzoon* isolates from European wildcats and the European lynx were analyzed, the three abovementioned separate species could be clearly delineated. This finding does not exclude that longer 18S RNA gene sequences may have the discriminatory power to distinguish between these species. However, it may exemplify that *cox* sequences may be better suited to detect more recent species splits than 18S rRNA gene sequences and facilitate the detection of cryptic species (Wang et al. 2017). *Cytauxzoon europaeus* was found in *F. silvestris* and *L. lynx* and corresponds to the *cox1-cox2* major EU1 haplotype identified in isolates from Germany, Italy, Czech Republic, Luxemburg, Romania, and Bosnia and Herzegovina. In contrast, *C. otratorum* and *C. banethi* correspond to the *cox1-cox2* minor EU2 and rare EU3 haplotype, respectively, which have been recovered from *F. silvestris* from Romania (Panait et al. 2021). At least four *Cytauxzoon* sp. isolates have been sequenced from the Iberian lynx (*Lynx pardinus*) (Millán et al. 2007; Meli et al. 2009). Based on their 18S rRNA gene, these isolates place into the *C. europaeus/C. otrantorum/C.*

banethi clade; however, *cox1-cox2* gene analysis might have the potential to reveal that they represent a cryptic additional novel *Cytauxzoon* species. Conversely, extended 18S rRNA gene sequences of *C. europaeus/C. otrantorum/C. banethi* may be able to distinguish parasites isolated from the Iberian lynx.

Reports on *Cytauxzoon* sp. findings in different locations and carnivore hosts, including domestic and wild cats in Brazil, meerkats in South Africa, Hokkaido bears in Japan, and Pallas' cats from Mongolia, indicate that the *Cytauxzoon* genus is far more geographically spread and diverse than initially expected (Ketz-Riley et al. 2003; André et al. 2009; Moustafa et al. 2020; Reichard et al. 2005; André et al. 2017; Furtado et al. 2017; de Sousa et al. 2018; Pedrassani et al. 2019; Sherrill and Cohn 2015; Leclaire et al. 2015; Wang et al. 2017; Moustafa et al. 2020). Accordingly, in a phylogenetic analysis of *Cytauxzoon* sp. 18S rRNA gene sequences, it has been demonstrated that the meerkat isolate occurred as a sister taxon to a large clade including at least five well-supported subclades: (i) *Cytauxzoon* sp. UR1; (ii) USA *C. felis* isolates; (iii) *Cytauxzoon* sp. isolated from ocelots (*Leopardus pardalis*) from Brazil; (iv) European isolates from domestic and wild cats; and (v) a *Cytauxzoon* species infecting Pallas' cats from Mongolia, defined as *C. manul* (Fig. 6, Panait et al. 2021).

It may therefore not be a surprise that an additional novel species designated *Cytauxzoon* sp. Kozhikode from domestic cats in India has just been reported (Malangmei et al. 2021). Most likely, this isolate represents a spillover infection from an indigenous not yet identified native cat species. Future investigations are needed to unravel the array of hosts that can harbor *Cytauxzoon* sp. infections, the extent of *Cytauxzoo*n intragenus diversity, and the pathogenic effects of different species in domestic cats and wild hosts.

Discussion

Since the discovery of the first piroplasmid by the turn of the twentieth century, a great number of species have been described as infecting wild and domestic hosts. Initially, species were described based on microscopic observations and the host from which they had been isolated. However, the unreliability and scarcity of morphological characteristics of hemoparasites have necessitated the use of molecular markers as characters for species identification, phylogenetic assessment, and taxonomy (Schnittger et al. 2012).

Traditionally, the 18S rRNA gene has been used as a phylogenetic and taxonomic marker and for species identification in piroplasmids and other hemoparasites (Greay et al. 2019). Several characteristics make the 18S rRNA gene a highly suitable marker across many taxonomic units. The 18S rRNA gene of eukaryotes possesses eight variable regions (V1 to V9, of which V6 displays low variability), highly appropriate for species identification. The V4 hypervariable region represents a fingerprint or barcode that is often exploited for the use of diagnostic PCR and PCR-based detection methods such as RLBH (reverse line blot hybridization; Gubbels et al. 1999; Schnittger et al. 2004; Criado-Fornelio 2007; Martínez-García et al. 2021). However, this highly variable region cannot be reliably aligned and its use may even result in artificial tree topologies, making it unsuitable for phylogenetic comparison. The remaining highly conserved 18S rRNA regions are appropriate for deep phylogeny, but as for any single marker gene, limitations exist, which may be best overcome by phylogenomics (Silva et al. 2015). Furthermore, mitochondrial *cox1* to *3* marker genes are more variable than conserved regions of 18S rRNA genes. They may therefore be assumed to increase resolution between species that could not be distinguished by 18S rRNA genes;

however, for the aforementioned reason, they are expected to exhibit lower resolution at deeper taxonomic levels and this supposition is supported by the demonstration of a high saturation rate of mitochondrial as compared to nuclear genes in *Plasmodium* (McIntosh et al. 1998; Silva et al. 2011, 2015).

When inferring phylogenetic trees, it is highly desirable to use complete or near-complete 18S rRNA genes since only then a reliable placement of an isolate can be achieved. As molecular phylogeny informs taxonomy, only then the latter will reflect the natural relationship of organisms, avoiding the representation of an artificial system. Molecular phylogeny of piroplasmids based on the 18S rRNA gene has identified at least six monophyletic lineages (Schnittger et al. 2012; Lack et al. 2012). The identity of the six piroplasmid lineages has been subsequently confirmed by alternative genomic approaches, such as the comparison of paralog expansion of the papain-like family of cysteine and serine rhomboid proteinases, deep sequencing, the use of mitochondrial genome markers, and the diversity of *N*-glycan biosynthesis pathways (Schreeg et al. 2016; Šlapeta et al. 2017; Ascencio et al. 2018; Florin-Christensen et al. 2021b; Gallenti et al. 2021). Most importantly, a phylogenomic approach using 90 nuclear- and 35 apicoplast-encoded genes resulted in a strongly supported similar phylogenetic topology when taxonomic units from Clade I and Clade III to VI were used (Muñoz-Gomez et al. 2019).

To the best of our knowledge, we have compiled in this study a summary of all piroplasmid species of farm and companion animals that have been described and/or characterized by molecular phylogenetic analysis using near-complete 18S rRNA or alternative reliable marker genes such as the *cox1* gene. In addition, isolates that are well-defined by molecular characterization, but still require a formal description, are also included (e.g., *Babesia* sp. Mymensingh; *Babesia* sp. Tengchong; *Babesia* sp. Xinjiang; *Babesia* sp. Akita 610; *Babesia* sp. Nigeria2014; *Babesia* sp. Coco; *Babesia* sp. Western Cape; *Theileria* sp. Yokohama; *Theileria* sp. Malawi; *Theileria* sp. OT3; *Theileria* sp. MK; *Theileria* sp. MSD; and *Cytauxzoon* sp. Kozhikide). In some cases, species names refer to a complex of isolates masking the real number of existing species (e.g., *T. orientalis*, *B. motasi*-like).

Overall, we present the existence of 55 species and/or well-defined isolates, and two species complexes (Table 7). By far the most piroplasmid species (15 species + 1 species complex) have been identified in cattle (including bovines, zebu, yak, and water buffalo). The second most frequent piroplasmid hosts are sheep and goats (12 species and one species complex). Interestingly, considerably more *Babesia* than *Theileria* spp. are found in cattle (9 vs. 6 + 1 species complex) than in small ruminants (4 + 1 species complex vs. 8). Noteworthy, all *Babesia* and *Theileria* spp. infecting farm animals belong to Clades V and VI. In contrast, *Theileria* spp. infecting the horse have been estimated to comprise at least six species, all belonging to Clade IV (Equus group; *T. equi*, *T. equi*-like), whereas only a single species of Clade VI (*Babesia* s.s.; *B. caballi*) has been reported.

Table 7. Inventory and classification of molecular characterized *Babesia*, *Theileria*, and *Cytauxzoon* species, well-defined isolates, and species complexes in farm and companion animals

	Babesia	*Theileria*	*Cytauxzoon*	Piroplasmida	Clade
Cattle	9	6 + 1[a]	0	15 + 1[a]	V, VI
Sheep and goat	4 + 1[a]	8	0	12 + 1[a]	V, VI
Horse	1	6	0	7	IV, VI
Dogs	9	0	0	9	Ib, II, VI
Cat	7	0	5	12	Id, II, III, VI
Σ	30 + 1[a]	20 + 1[a]	5	55 + 2[a]	

[a]Number of species complexes

In contrast to farm animals and equids, dogs are exclusively infected by *Babesia* spp. (nine species), whereas cats are infected by *Babesia* spp. (seven species) and *Cytauxzoon* spp. (five species); furthermore, *Cytauxzoon* species have not been identified in any farm or companion animal other than cats. It is worthy to note that piroplasmids infecting dogs and cats segregate into three (Clades VI, II, Ib) and four (Clades VI, III, II, Id) distinct monophyletic lineages, respectively. Based on this observation, we hypothesize that carnivores represent earlier vertebrate hosts of piroplasmids than ruminants and equines, which corresponds with the earlier evolutionary origin of Canidae (≈ 40 mya) and Felidae (≈ 25 mya) compared to that of Bovidae (≈ 20 mya) (Bovini, cattle ≈ 13 mya); sheep and goat (Caprini, ≈ 9 mya); and the modern horse (Equus, ≈ 5 mya) (Wang and Tedford 2008; Werdelin et al. 2010; Hassanin et al. 2012; Jiang et al. 2014; Chen et al. 2019; Librado and Orlando 2021).

It is remarkable that exclusively *Babesia* s.s. parasites have conquered all species of farm and companion animals as hosts. In this context, it must be emphasized that *Babesia* s.s. are unique among hemoparasites and represent the only group of piroplasmids (Clade VI) that have developed transovarial transmission. This feature has turned the long-term strategy of piroplasmid survival upside down. This is best exemplified by *T. equi* and *B. caballi* infecting the horse, which are transmitted transstadially and transovarially, respectively. For *T. equi* transmission, tick larvae must first feed on a *T. equi*-infected horse to be able to transmit the parasite subsequently to an uninfected horse, with the possible outcome that *T. equi* establishes itself in the horse population. Therefore, the key survival strategy for this piroplasmid is to promote a lifelong carrier status of the horse to ensure infection of newly born ticks. In contrast, in the case of the transovarially transmitted *B. caballi*, the parasite will be passed on into the next tick generation once a tick is infected, without the need for prior feeding on an infected horse. Thus, for *B. caballi*, the tick functions as a carrier, so this species does not depend on a prolonged carrier status of the horse for transmission into the next generation, and its establishment in a vertebrate host population is greatly facilitated. This assumption is corroborated by the worldwide collection of experimental data comparing molecular (presence of the parasite in the host) and seroprevalence (parasite exposure of the host) of *T. equi* and *B. caballi* infection of the horse. The molecular and seroprevalence rates of *T. equi* infection of horses are similar (34.6% vs. 33.2%), whereas the molecular prevalence of *B. caballi* infection is consistently three times lower than its seroprevalence in diverse geographic areas (7.4% vs. 20.5% in average) (Tirosh-Levy et al. 2020). From what has been outlined, it becomes clear that host switching might be significantly facilitated by transovarial transmission, explaining why it is relatively frequently observed in Clade VI piroplasmids, but absent in Clades III, IV, and V, and infrequent in the remaining piroplasmid lineages Clades I and II (Figs. 2, 3, 6, and 7).

Our study reveals that a considerable number of different piroplasmid species may regularly infect a single host species. This is not restricted to farm and companion animals but is also regularly observed in wild animal hosts (e.g., Leclaire et al. 2015; Yabsley et al. 2017; Garrett et al. 2019; Moustafa et al. 2020). Therefore, it can be extrapolated that the number of piroplasmid species that exist is considerably larger than the number of vertebrate host species they infect. Multiple species infecting a single vertebrate host may belong to different piroplasmid lineages (e.g., *C. felis* and *B. felis* infecting cats or *T. equi* and *B. caballi* infecting the horse) but may also belong to the same piroplasmid lineage (e.g., *B. bovis* and *B. bigemina*, or different MPSP types of the *T. orientalis* sp. complex infecting cattle) (Jenkins et al. 2015; Alvarado-Rybak et al. 2016; Romero-Salas et al. 2016; Li et al. 2020; Penzhorn and Oosthuizen 2020). Furthermore, piroplasmid species infecting the same host may be endemic in the same region (e.g., *B. bovis* and *B. bigemina* in bovines; *T. equi* and *T. haneyi* in the horse), but they may also show very distinct geographic distribution patterns (e.g., *B. bovis* and *B. divergens* in cattle; *C. felis* and *B. leo* in cats) (Zintl et al. 2003; Alvarado-Rybak et al. 2016; Onyiche et al. 2019; Tirosh-Levy et al. 2020). Observed geographic distribution patterns of piroplasmid species are complex since the ecological needs of both the mammalian host and the vector tick must be met (Zimmermann et al. 2021).

It is commonly acknowledged that the observation of specific piroplasmid DNA in a tick does not mean that it represents a competent vector. Following the same argument, the identification of specific piroplasmid DNA in a vertebrate host may not mean that the parasite finalizes its developmental cycle with this host. The latter phenomenon has been studied in cross-infection experiments for *T. annulata* and *T. lestoquardi*, which infect cattle and small ruminants, respectively. Both piroplasmid species are very closely related; however, although *T. annulata* can infect sheep, it does not complete its development into the piroplasmid parasite stage. Thus, after infection, *T. annulata* DNA can be detected in sheep, though its life cycle will not be completed, and it will not become established in this host. On the other hand, infection of cattle with the sheep-infecting *T. lestoquardi* could not be achieved, suggesting a strict host specificity for this species (Leemans et al. 1998, 1999).

Increasingly, infections in unexpected hosts are reported and it may be assumed that human interventions favor this observation (Schnittger et al. 2012; Uilenberg et al. 2018; Bishop et al. 2020; Penzhorn and Oosthuizen 2020). Farm and companion animals are commonly raised in close proximity, and it is to be assumed that this strongly promotes spillover or accidental infections (Schnittger and Florin-Christensen 2018). It is preferable to interpret an accidental infection as an infrequent transmission that may or may not result in the immediate successful infection of an individual host and, in a longer period, possibly in the continuous establishment of a piroplasmid species in a new vertebrate and/or tick host. Thus, it represents an incipient evolutionary process of adaptation of a piroplasmid species to a potential tick-vertebrate host system. If all necessary factors are favorable, an accidental infection may finally result in the successful establishment of the piroplasmid parasite in the tick-vertebrate host system.

Although ticks typically feed on a wide range of hosts and, correspondingly, piroplasmid DNA is commonly found in unexpected hosts, phylogenetic analysis strongly suggests that a host switch that results in successful prolonged establishment of a piroplasmid species in a tick-vertebrate host system is an utterly rare event. When scrutinizing phylogenetic trees, in many piroplasmid lineages, evolution by cospeciation seems to prevail and, as mentioned above, host switch is, on an evolutionary time scale, only observed in *Babesia* s.s. (Clade VI), yet it is either a completely absent or an extremely rare event in all other piroplasmid lineages

(Figs. 2 and 3, Jalovecka et al. 2019). Interestingly, host switch between carnivore and prey animals is virtually absent, a finding that seems to be counterintuitive given the periodic close encounter over a huge span of evolutionary time between these two groups of animals (Fig. 3, carnivore clades A to D). It may be assumed that the piroplasmid-tick-vertebrate triad represents a highly complex system in which a considerable number of biotic and abiotic factors must be met before a successful prolonged establishment of piroplasmid transmission is finally achieved.

Although the 18S rRNA gene is a reliable marker for the identification of piroplasmid species, several novel species have been identified using additional molecular markers. Panait et al. (2021) could not resolve *Cytauxzoon* spp. in wild cats and European lynx using 1000- to 1200-bp-long 18S rRNA gene sequences but could distinguish and describe *C. europaeus*, *C. otratorum*, and *C. banethi* when using the *cox1* and *cox2* marker genes. The 18S rRNA sequence results could indicate that these parasite species represent cryptic species; however, it cannot be ruled out that longer 18S rRNA gene sequences would be able to distinguish these three parasite species. The 18S rRNA genes could distinguish *Theileria* sp. Yokoyama and *Babesia* sp. Mymensingh from the closely related *T. annulata* and *B. bigemina*, respectively. For an improved delineation of *Theileria* sp. Yokoyama, Sivakumar et al. (2019, 2020) used the highly variable surface protein-encoding genes *tams1* (merozoite-piroplasm surface antigen-1) and *tasp* (*T. annulata* surface protein), while for *Babesia* sp. Mymensingh, the more variable *ama-1* (apical membrane antigen 1) and *cox3* gene was used to corroborate their findings. However, *tams1*, *tasp*, and *ama-1* genes are exclusively present in very closely related taxons of these species and are therefore not applicable to identify and differentiate other distantly related piroplasmid isolates.

Increasingly, the use of deposited sequences for isolate identification and bioinformatic analysis is becoming cumbersome and complicated. Whenever possible, it is highly preferable to generate full-length 18S rRNA gene sequences as only this allows a confident inference of phylogenetic relations and the determination to which species or clade a given isolate belongs to. When depositing sequences in the GenBank, it is highly desirable to give them a single unique isolate designation, which in some cases is lacking, which complicates referral to them before a formal species description is available (e.g., Leclaire et al. 2015). In our view, the scientific community of piroplasmid researchers would benefit from avoiding a continuous change in the designation of an isolate but be consistent in its use as otherwise this results in confusion and complicates scientific communication (Baneth et al. 2015, 2019). As an example, inconsistent use of designations has been observed for the *B. motasi*-like group infecting sheep, which makes it extremely difficult to approach this research field (Liu et al. 2007; Niu et al. 2016). Furthermore, annotation of sequences with species names is often done without certainty, leading to confusing sequence designations that may lead to artificial studies when sequences are not highly critically evaluated. Importantly, the formal species description of isolates should be encouraged as in many cases the required biological information is available (e.g., *Cytauxzoon* and *Babesia* isolated from meerkat, Leclaire et al. 2015).

Sequences that have been obtained by cloning after amplification with *Taq* polymerase are more prone to errors than when polymerases with proofreading activity are used. The informational background noise is continuously increasing through an accumulation of such sequences in databases, and the risk of the generation of artificial research results is increased when such sequences are included in phylogenetic analyses, studies of molecular evolution, and/or searched by BLAST. This is not a forecast of the future but the report of artifactual or

misdesignated sequences is not uncommon and the deposit of sequences that contain sequence errors is steadily increasing (e.g., Man et al. 2016, the reported 18S RNA gene sequence of *Babesia* sp. XXB/HangZhou likely originates from the fungi *Cladosporidium*; Malandrin 2021, the 18S RNA gene of *B. bennetti* represents highly likely an artificial chimeric sequence). This observation is not limited to piroplasmid research, but the growing report of artifactual research results due to sequence errors is already becoming increasingly evident in human genetics research (Else 2021; Park et al. 2021). It needs to be considered that databases are only as good as the data they contain and only members of the scientific community can prevent their deterioration by taking responsibility to keep them highly accurate.

As has been recently emphasized by Penzhorn and Oosthuizen (2020), to refer to all piroplasmids that infect cats as *B. felis* is scientifically irresponsible. This argument extends to *Cytauxzoon* spp. identified in cats from Europe, which should not be referred to as *C. felis*, unless identification of this species has been demonstrated by molecular analysis. Another example in this regard may refer to isolates of the Equus group (Clade IV). Currently, all *Theileria* isolates from the horse, and other hosts, that segregate into this clade are indiscriminately referred to as *T. equi*; however, it should be borne in mind that by this habit, the scientific name *T. equi* becomes increasingly meaningless. Recently, isolates from tapir (*Tapirus terrestris*) have been referred to as *T. equi*. However, the fact that these isolates place as a sister group to all other isolates of the Equus group strongly suggests that they represent a novel species; it may have been therefore preferable to refer to them either as *T. equi*-like or give them an alternative isolate designation (de Souza Gonçalves et al. 2020).

Concerns of piroplasmid infections of domestic animals are especially related to direct and indirect productive losses in the case of bovids and small ruminants; decreased performance and restrictions to international travel in the case of equids; and animal welfare in the case of companion animals, the latter of which applies to all animal species. Currently, the tools to combat these infections are scarce and/or need improvement. Chemical acaricides contaminate the environment and create resistance (Mosqueda et al. 2012). Vaccines only target a handful of piroplasmids and have a limited distribution, and the most used drugs to combat these parasites in livestock leave residues in meat and milk (Coldham et al. 1995; Florin-Christensen et al. 2014). In a scenario of global warming in which ticks are extending their geographical ranges, new measures are urgently needed to control the deleterious effects caused by piroplasmids in domestic animals (Inci et al. 2016, Nicaretta et al. 2021). As to our knowledge, the presently known piroplasmid species, isolates, and species complexes of farm and companion animals have been presented in this study and it can be foreseen that novel species and phylogenetic lineages of this group of parasites will emerge.

Contributions

Conceptualization: LS; formal analysis: LS and MFC; investigation: DO, LS, and MFC; writing—original draft: MFC, SG, RB, and LS; writing—review and editing: RB, AN, DO, MFC, and LS; visualization: SG and LS; supervision: LS; funding acquisition: LS and MFC

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Conflict of interest

The authors declare no competing interests.

References

Acici M, Umur S, Guvenc T, Arslan HH, Kurt M (2008) Seroprevalence of equine babesiosis in the Black Sea region of Turkey. Parasitol Int 57(2):198–200

Agina OA, Shaari MR, Isa NMM, Ajat M, Zamri-Saad M, Hamzah H (2020) Clinical pathology, immunopathology and advanced vaccine technology in bovine theileriosis: a review. Pathogens 9:697. https://doi.org/10.3390/pathogens9090697

Ahmed JS, Schnittger L, Mehlhorn H (1999) Review: *Theileria* schizonts induce fundamental alterations in their host cells. Parasitol Res 85:527–538. https://doi.org/10.1007/s004360050592

Ahmed JS, Luo J, Schnittger L, Seitzer U, Jongejan F, Yin H (2006) Phylogenetic position of small-ruminant infecting piroplasms. Ann N Y Acad Sci 1081:498–504. https://doi.org/10.1196/annals.1373.074

Alani AJ, Herbert IV (1988) Pathogenesis of infection with *Theileria recondita* (Wales) isolated from *Haemaphysalis punctata* from north Wales. Vet Parasitol 28:293–301. https://doi.org/10.1016/0304-4017(88)90076-3

Alho AM, Silva J, Fonseca MJ, Santos F, Nunes C, de Carvalho LM, Rodrigues M, Cardoso L (2016) First report of *Cytauxzoon* sp. infection in a domestic cat from Portugal. Parasit Vectors 9:220. https://doi.org/10.1186/s13071-016-1506-5

Allred DR (2019) J variable and variant protein multigene families in Babesia bovis persistence. Pathogens. 8:76. https://doi.org/10.3390/pathogens8020076

Allsopp MT, Allsopp BA (2006) Molecular sequence evidence for the reclassification of some Babesia species. Ann N Y Acad Sci 1081:509–517. https://doi.org/10.1196/annals.1373.076

Altay K, Dumanli N, Aktas M (2007) Molecular identification, genetic diversity and distribution of *Theileria* and *Babesia* species infecting small ruminants. Vet Parasitol 147:161–165. https://doi.org/10.1016/j.vetpar.2007.04.001

Alvarado-Rybak M, Solano-Gallego L, Millán J (2016) A review of piroplasmid infections in wild carnivores worldwide: importance for domestic animal health and wildlife conservation. Parasit Vectors 9:538. https://doi.org/10.1186/s13071-016-1808-7

André MR, Adania CH, Machado RZ, Allegretti SM, Felippe PA, Silva KF, Nakaghi AC, Dagnone AS (2009) Molecular detection of Cytauxzoon spp. in asymptomatic Brazilian wild captive felids. J Wildl Dis 45:234–237. https://doi.org/10.7589/0090-3558-45.1.234

André MR, Baccarim Denardi NC, Marques de Sousa KC, Gonçalves LR, Henrique PC, Grosse Rossi Ontivero CR, Lima Gonzalez IH, Cabral Nery CV, Fernandes Chagas CR, Monticelli C, Alexandre de Santis AC, Machado RZ (2014) Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil. Ticks Tick Borne Dis 5:545–551. https://doi.org/10.1016/j.ttbdis.2014.03.011

André MR, Filgueira KD, Calchi AC, Sousa KCM, Gonçalves LR, Medeiros VB, Ximenes PA, Lelis ICNG, Meireles MVN, Machado RZ (2017) Co-infection with arthropod-borne pathogens in domestic cats. Rev Bras Parasitol Vet 26:525–531. https://doi.org/10.1590/S1984-29612017064

André MR, Herrera HM, Fernandes Sde J, de Sousa KC, Gonçalves LR, Domingos IH, de Macedo GC, Machado RZ (2015) Tick-borne agents in domesticated and stray cats from the city of Campo Grande, state of Mato Grosso do Sul, midwestern Brazil. Ticks Tick Borne Dis 6:779–786. https://doi.org/10.1016/j.ttbdis.2015.07.004

Ascencio ME, Florin-Christensen M, Mamoun CB, Weir W, Shiels B, Schnittger L (2018) Cysteine proteinase C1A paralog profiles correspond with phylogenetic lineages of pathogenic piroplasmids. Vet Sci 5:41. https://doi.org/10.3390/vetsci5020041

Azagi T, Jaarsma RI, Docters van Leeuwen A, Fonville M, Maas M, Franssen FFJ, Kik M, Rijks JM, Montizaan MG, Groenevelt M, Hoyer M, Esser HJ, Krawczyk AI, Modrý D, Sprong H, Demir S (2021) Circulation of *Babesia* species and their exposure to humans through *Ixodes ricinus*. Pathogens 10:386. https://doi.org/10.3390/pathogens10040386

Aziz KJ, Al-Barwary LT (2020) Molecular identification of *Theileria equi* and *Babesia caballi* from ixodid ticks infesting equids in Erbil province, northern of Iraq. Adv Anim Vet Sci 8:1286–1296

Babes V (1892) L'etiologie d'une enzootie des moutons, denommee Carceag en Roumanie. (The etiology of an enzootic disease of sheep, called Carceag, in Romania) (in French). C R Hebd Acad Sci 115:359–361

Baneth G (2018a) *Babesia* of domestic dogs. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 241–258

Baneth G (2018b) Antiprotozoal treatment of canine babesiosis. Vet Parasitol 254:58–63. https://doi.org/10.1016/j.vetpar.2018.03.001

Baneth G, Cardoso L, Brilhante-Simões P, Schnittger L (2019) Establishment of *Babesia vulpes* n. sp. (Apicomplexa: Babesiidae), a piroplasmid species pathogenic for domestic dogs. Parasit Vectors 12:129. https://doi.org/10.1186/s13071-019-3385-z

Baneth G, Florin-Christensen M, Cardoso L, Schnittger L (2015) Reclassification of *Theileria annae* as *Babesia vulpes* sp. nov. Parasit Vectors 8:207. https://doi.org/10.1186/s13071-015-0830-5

Baneth G, Kenny MJ, Tasker S, Anug Y, Shkap V, Levy A, Shaw SE (2004) Infection with a proposed new subspecies of *Babesia canis, Babesia canis* subsp. presentii, in domestic cats. J Clin Microbiol 42:99–105. https://doi.org/10.1128/JCM.42.1.99-105.2004

Baneth G, Nachum-Biala Y, Birkenheuer AJ, Schreeg ME, Prince H, Florin-Christensen M, Schnittger L, Aroch I (2020) A new piroplasmid species infecting dogs: morphological and molecular characterization and pathogeny of *Babesia negevi* n. sp. Parasit Vectors 13:130. https://doi.org/10.1186/s13071-020-3995-5

Barbosa AD, Austen J, Portas TJ, Friend JA, Ahlstrom LA, Oskam CL, Ryan UM, Irwin PJ (2019) Sequence analyses at mitochondrial and nuclear loci reveal a novel *Theileria* sp. and aid in the phylogenetic resolution of piroplasms from Australian marsupials and ticks. PLoS One 14:e0225822. https://doi.org/10.1371/journal.pone.0225822

Bawm S, Myaing TT, Thu MJ, Akter S, Htun LL, Win MM, Nonaka N, Nakao R, Katakura K (2021) PCR detection and genetic characterization of piroplasms from dogs in Myanmar, and a possible role of dogs as reservoirs for *Theileria* parasites infecting cattle, water buffaloes, and goats. Ticks Tick Borne Dis 12:101729. https://doi.org/10.1016/j.ttbdis.2021.101729

Beck R, Vojta L, Mrljak V, Marinculic A, Beck A, Zivicnjak T et al (2009) Diversity of Babesia and Theileria species in symptomatic and asymptomatic dogs in Croatia. Int J Parasitol 39:843–848

Benitez D, Cetrá B, Florin-Christensen M (2012) *Rhipicephalus (Boophilus) microplus* ticks can complete their life cycle on the water buffalo (*Bubalus bubalis*). J Buffalo Sci 1:193–197

Benitez D, Mesplet M, Echaide I, Torioni de Echaide S, Schnittger L, Florin-Christensen M (2018) Mitigated clinical disease in water buffaloes experimentally infected with *Babesia bovis*. Ticks Tick Borne Dis 9:1358–1363. https://doi.org/10.1016/j.ttbdis.2018.04.012

Bhoora R, Buss P, Guthrie AJ, Penzhorn BL, Collins NE (2010) Genetic diversity of piroplasms in plains zebra (Equus quagga burchellii) and Cape mountain zebra (Equus zebra zebra) in South Africa. Vet Parasitol 174:145–149. https://doi.org/10.1016/j.vetpar.2010.08.014

Bhoora RV, Collins NE, Schnittger L, Troskie C, Marumo R, Labuschagne K, Smith RM, Dalton DL, Mbizeni S (2020) Molecular genotyping and epidemiology of equine piroplasmids in South Africa. Ticks Tick Borne Dis 11:101358. https://doi.org/10.1016/j.ttbdis.2019.101358

Bhoora R, Franssen L, Oosthuizen MC, Guthrie AJ, Zweygarth E, Penzhorn BL, Jongejan F, Collins NE (2009) Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. Vet Parasitol 159:112–120. https://doi.org/10.1016/j.vetpar.2008.10.004

Biasibetti E, Sferra C, Lynen G, Di Giulio G, De Meneghi D, Tomassone L, Valenza F, Capucchio MT (2016) Severe meningeal fibrinoid vasculitis associated with *Theileria taurotragi* infection in two short-horned Zebu cattle. Trop Anim Health Prod 48:1297–1299. https://doi.org/10.1007/s11250-016-1072-z

Bimbi P (1916) Equine piroplasmosis in Sardinia. Moderno Zooiatro. Bologna, Parte Scient 5:225–233

Birkenheuer AJ, Buch J, Beall MJ, Braff J, Chandrashekar R (2020) Global distribution of canine *Babesia* species identified by a commercial diagnostic laboratory. Vet Parasitol Reg Stud Reports 22:100471. https://doi.org/10.1016/j.vprsr.2020.100471

Birkenheuer AJ, Neel J, Ruslander D, Levy MG, Breitschwerdt EB (2004) Detection and molecular characterization of a novel large *Babesia* species in a dog. Vet Parasitol 124:151–160. https://doi.org/10.1016/j.vetpar.2004.07.008

Bishop RP, Kappmeyer LS, Onzere CK, Odongo DO, Githaka N, Sears KP, Knowles DP, Fry LM (2020) Equid infective *Theileria* cluster in distinct 18S rRNA gene clades comprising multiple taxa with unusually broad mammalian host ranges. Parasit Vectors 13:261. https://doi.org/10.1186/s13071-020-04131-0

Blouin E, Kocan AA, Glenn BL, Kocan KM (1984) Transmisssion of Cytauxzoon felis Kier, 1979 from bobcats, Felis rufus (Schreber), to domestic cats by Dermacentor variabilis (Say). J Wildl Dis 20:241–242

Blouin EF, van Rensburg L (1988) An ultrastructural study of the development of *Babesia occultans* in the salivary glands of adult *Hyalomma marginatum* rufipes. Onderstepoort J Vet Res 55:93–100

Bock R, Jackson L, de Vos A, Jorgensen W (2004) Babesiosis of cattle. Parasitology 129:S247–S269. https://doi.org/10.1017/s0031182004005190

Bogema DR, Micallef ML, Liu M, Padula MP, Djordjevic SP, Darling AE, Jenkins C (2018) Analysis of *Theileria orientalis* draft genome sequences reveals potential species-level divergence of the Ikeda, Chitose and Buffeli genotypes. BMC Genomics 19:298. https://doi.org/10.1186/s12864-018-4701-2

Bosman AM, Oosthuizen MC, Peirce MA, Venter EH, Penzhorn BL (2010) *Babesia lengau* sp. nov., a novel *Babesia* species in cheetah (Acinonyx jubatus, Schreber, 1775) populations in South Africa. J Clin Microbiol 48:2703–2708. https://doi.org/10.1128/JCM.02266-09

Bosman AM, Oosthuizen MC, Venter EH, Steyl JC, Gous TA, Penzhorn BL (2013) *Babesia lengau* associated with cerebral and haemolytic babesiosis in two domestic cats. Parasit Vectors 6:128. https://doi.org/10.1186/1756-3305-6-128

Bosman AM, Penzhorn BL, Brayton KA, Schoeman T, Oosthuizen MC (2019) A novel *Babesia* sp. associated with clinical signs of babesiosis in domestic cats in South Africa. Parasit Vectors 12:138. https://doi.org/10.1186/s13071-019-3395-x

Bosman AM, Venter EH, Penzhorn BL (2007) Occurrence of *Babesia felis* and *Babesia leo* in various wild felid species and domestic cats in Southern Africa, based on reverse line blot analysis. Vet Parasitol 144:33–38. https://doi.org/10.1016/j.vetpar.2006.09.025

Braga MDSCO, Costa FN, Gomes DRM, Xavier DR, André MR, Gonçalves LR, Freschi CR, Machado RZ (2017) Genetic diversity of piroplasmids species in equids from island of São Luís, northeastern Brazil. Rev Bras Parasitol Vet 26:331–339. https://doi.org/10.1590/S1984-29612017046

Byaruhanga C, Collins NE, Knobel D, Chaisi ME, Vorster I, Steyn HC, Oosthuizen MC (2016) Molecular investigation of tick-borne haemoparasite infections among transhumant zebu cattle in Karamoja Region, Uganda. Vet Parasitol Reg Stud Reports 3-4:27–35. https://doi.org/10.1016/j.vprsr.2016.06.004

Carini A, Maciel J (1914) Sobre a moléstia dos cães, chamada Nambi-Uvú, e o seu parasita (*Rangelia vitalii*). An Paul Med Cir 3:65–71

Carli E, Trotta M, Chinelli R, Drigo M, Sinigoi L, Tosolini P, Furlanello T, Millotti A, Caldin M, Solano-Gallego L (2012) *Cytauxzoon* sp. infection in the first endemic focus described in domestic cats in Europe. Vet Parasitol 183:343–352. https://doi.org/10.1016/j.vetpar.2011.07.025

Carret C, Walas F, Carcy B, Grande N, Précigout E, Moubri K, Schetters TP, Gorenflot A (1999) *Babesia canis canis*, *Babesia canis vogeli*, *Babesia canis rossi*: differentiation of the three subspecies by a restriction fragment length polymorphism analysis on amplified small subunit ribosomal RNA genes. J Eukaryot Microbiol 46:298–303. https://doi.org/10.1111/j.1550-7408.1999.tb05128.x

Chae JS, Allsopp BA, Waghela SD, Park JH, Kakuda T, Sugimoto C, Allsopp MT, Wagner GG, Holman PJ (1999) A study of the systematics of *Theileria* spp. based upon small-subunit ribosomal RNA gene sequences. Parasitol Res 85:877–883. https://doi.org/10.1007/s004360050651

Chaisi ME, Collins NE, Potgieter FT, Oosthuizen MC (2013) Sequence variation identified in the 18S rRNA gene of *Theileria mutans* and *Theileria velifera* from the African buffalo (*Syncerus caffer*). Vet Parasitol 191:132–137. https://doi.org/10.1016/j.vetpar.2012.08.005

Chatanga E, Kainga H, Maganga E, Hayashida K, Katakura K, Sugimoto C, Nonaka N, Nakao R (2021) Molecular identification and genetic characterization of tick-borne pathogens in sheep and goats at two farms in the central and southern regions of Malawi. Ticks Tick Borne Dis 12:101629. https://doi.org/10.1016/j.ttbdis.2020.101629

Chauvin A, Moreau E, Bonnet S, Plantard O, Malandrin L (2009) *Babesia* and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. Vet Res 40:37. https://doi.org/10.1051/vetres/2009020

Chen L, Qiu Q, Jiang Y, Wang K, Lin Z, Li Z et al (2019) Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. Science 364(6446):eaav6202. https://doi.org/10.1126/science.aav6202

Coldham NG, Moore AS, Dave M, Graham PJ, Sivapathasundaram S, Lake BG, Sauer MJ (1995) Imidocarb residues in edible bovine tissues and in vitro assessment of imidocarb metabolism and cytotoxicity. Drug Metab Dispos 23:501–505

Cooke BM, Mohandas N, Cowman AF, Coppel RL (2005) Cellular adhesive phenomena in apicomplexan parasites of red blood cells. Vet Parasitol 132:273–295. https://doi.org/10.1016/j.vetpar.2005.07.002

Coultous RM, Phipps P, Dalley C, Lewis J, Hammond TA, Shiels BR, Weir W, Sutton DGM (2019) Equine piroplasmosis status in the UK: an assessment of laboratory diagnostic submissions and techniques. Vet Rec 184:95. https://doi.org/10.1136/vr.104855

Criado-Fornelio A (2007) A review of nucleic-acid-based diagnostic tests for *Babesia* and *Theileria*, with emphasis on bovine piroplasms. Parassitologia 49(Suppl 1):39–44

Criado-Fornelio A, Buling A, Pingret JL, Etievant M, Boucraut-Baralon C, Alongi A, Agnone A, Torina A (2009) Hemoprotozoa of domestic animals in France: prevalence and molecular characterization. Vet Parasitol 159:73–76. https://doi.org/10.1016/j.vetpar.2008.10.012

Criado-Fornelio A, Gónzalez-del-Río MA, Buling-Saraña A, Barba-Carretero JC (2004) The "expanding universe" of piroplasms. Vet Parasitol 119:337–345. https://doi.org/10.1016/j.vetpar.2003.11.015

Dahmana H, Amanzougaghene N, Davoust B, Normand T, Carette O, Demoncheaux JP, Mulot B, Fabrizy B, Scandola P, Chik M, Fenollar F, Mediannikov O (2019) Great diversity of Piroplasmida in Equidae in Africa and Europe, including potential new species. Vet Parasitol Reg Stud Reports 18:100332. https://doi.org/10.1016/j.vprsr.2019.100332

de la Fuente J, Almazán C, Canales M, Pérez de la Lastra JM, Kocan KM, Willadsen P (2007) A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Anim Health Res Rev 8:23–28. https://doi.org/10.1017/S1466252307001193

De Lorenzo C, Boabaid FM, de Oliveira LGS, Bianchi MV, Félix ML, Armúa-Fernández MT, Soares JF, Venzal JM, Sonne L (2021) *Rangelia vitalii* in free-living crab-eating foxes (*Cerdocyon thous*) in Uruguay. Ticks Tick Borne Dis 12:101765. https://doi.org/10.1016/j.ttbdis.2021.101765

de Sousa KCM, Fernandes MP, Herrera HM, Freschi CR, Machado RZ, André MR (2018) Diversity of piroplasmids among wild and domestic mammals and ectoparasites in Pantanal wetland, Brazil. Ticks Tick Borne Dis 9:245–253. https://doi.org/10.1016/j.ttbdis.2017.09.010

de Souza Gonçalves T, de Nazaré Leite Barros F, Inoue LS, de Farias DM, Dos Santos Lima J, Nobre AV, Azenha Aidar ES, Ferreira Diniz RR, Gering AP, Scofield A (2020) Natural *Theileria equi* infection in captive *Tapirus terrestris* (Perissodactyla: Tapiridae) in the Brazilian Amazon. Ticks Tick Borne Dis 11:101452. https://doi.org/10.1016/j.ttbdis.2020.101452

De Vos AJ, Bessenger R, Banting LF (1981) *Theileria taurotragi*: a probable agent of bovine cerebral theileriosis. Onderstepoort J Vet Res 48(3):177–178

de Waal DT (1992) Equine piroplasmosis: a review. Br Vet J 148:6–14. https://doi.org/10.1016/0007-1935(92)90061-5

Decaro N, Larocca V, Parisi A, Losurdo M, Lia RP, Greco MF, Miccolis A, Ventrella G, Otranto D, Buonavoglia C (2013) Clinical bovine piroplasmosis caused by *Babesia occultans* in Italy. J Clin Microbiol 51:2432–2434. https://doi.org/10.1128/JCM.00713-13

Dhaygude VS, Kundu K, Kamdi BP, Bagal UR, Bhosale SB, Sabharwal D (2021) Investigations on first confirmed outbreak of ovine theileriosis (Theileria luwenshuni) from Maharashtra State, India. Indian J Anim Res 55:951–955. https://doi.org/10.1051/parasite/2011184311

Eamens GJ, Gonsalves JR, Jenkins C, Collins D, Bailey G (2013) *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. Vet Parasitol 191:209–217. https://doi.org/10.1016/j.vetpar.2012.09.007

El Imam AH, Hassan SM, Gameel AA, El Hussein AM, Taha KM, Salih DA (2015) Variation in susceptibility of three Sudanese sheep ecotypes to natural infection with *Theileria lestoquardi*. Small Rumin Res 124:105–111

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

Else H (2021) Errors in genetic sequences mar hundreds of studies, https://www.nature.com/articles/d41586-021-02136-y

Estrada-Peña A, Venzal JM, Nava S, Mangold A, Guglielmone AA, Labruna MB, de la Fuente J (2012) Reinstatement of *Rhipicephalus (Boophilus) australis* (Acari: Ixodidae) with redescription of the adult and larval stages. J Med Entomol 49:794–802. https://doi.org/10.1603/me11223

Ferreri L, Benitez D, Dominguez M, Rodriguez A, Asenzo G, Mesplet M, Florin-Christensen M, Schnittger L (2008) Water buffalos as carriers of *Babesia bovis* in Argentina. Ann N Y Acad Sci 1149:149–151. https://doi.org/10.1196/annals.1428.036

Ferris DH (1979) A progress report on the status of a new disease of American cats: cytauxzoonosis. Comp Immunol Microbiol Infect Dis 1:269–276. https://doi.org/10.1016/0147-9571(79)90028-6

Florin-Christensen M, Rodriguez AE, Suárez CE, Ueti MW, Delgado FO, Echaide I, Schnittger L (2021b) N-Glycosylation in piroplasmids: diversity within simplicity. Pathogens 10:50. https://doi.org/10.3390/pathogens10010050

Florin-Christensen M, Schnittger L (2009) Piroplasmids and ticks: a long-lasting intimate relationship. Front Biosci (Landmark Ed) 14:3064–3073. https://doi.org/10.2741/3435

Florin-Christensen M, Schnittger L, Bastos RG, Rathinasamy VA, Cooke VM, Alzan HF, Suarez CE (2021a) Pursuing effective vaccines against cattle diseases caused by apicomplexan protozoa. CAB Reviews 16, No. 024

Florin-Christensen M, Suarez CE, Rodriguez AE, Flores DA, Schnittger L (2014) Vaccines against bovine babesiosis: where we are now and possible roads ahead. Parasitology 28:1–30. https://doi.org/10.1017/S0031182014000961

Fredo G, Bianchi MV, De Andrade CP, De Souza SO, Leite-Filho RV, Bandinelli MB, Amorim DB, Driemeier D, Sonne L (2015) Natural infection of wild canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) with the intraendothelial piroplasm *Rangelia vitalii* in southern Brazil. J Wildl Dis 51:880–884. https://doi.org/10.7589/2014-12-283

Freyburger L, Lemaitre L, Médaille C, Oberli F, Fanchon L, Bergamo P (2011) Comparative safety study of two commercialised vaccines against canine babesiosis induced by *Babesia canis*. Parasite 18:311–318. https://doi.org/10.1051/parasite/2011184311

Fry LM, Bastos RG, Stone BC, Williams LB, Knowles DP, Murphy SC (2019) Gene gun DNA immunization of cattle induces humoral and CD4 T-cell-mediated immune responses against the *Theileria parva* polymorphic immunodominant molecule. Vaccine 37:1546–1553. https://doi.org/10.1016/j.vaccine.2019.02.009

Fukumoto S, Suzuki H, Igarashi I, Xuan X (2005) Fatal experimental transplacental *Babesia gibsoni* infections in dogs. Int J Parasitol 35:1031–1035. https://doi.org/10.1016/j.ijpara.2005.03.018

Furtado MM, Taniwaki SA, Metzger B, Dos Santos PK, O'Dwyer HL, de Almeida Jácomo AT, Porfírio GEO, Silveira L, Sollmann R, Tôrres NM, Ferreira Neto JS (2017) Is the free-ranging jaguar (*Panthera onca*) a reservoir for *Cytauxzoon felis* in Brazil? Ticks Tick Borne Dis 8:470–476. https://doi.org/10.1016/j.ttbdis.2017.02.005

Gallenti R, Poklepovich T, Florin-Christensen M, Schnittger L (2021) The repertoire of serine rhomboid proteases of piroplasmids of importance to animal and human health. Int J Parasitol 51:455–462. https://doi.org/10.1016/j.ijpara.2020.10.010

Ganzinelli S, Rodriguez AE, Schnittger L, Florin-Christensen M (2018) *Babesia* of domestic ruminants. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 215–239

Garrett KB, Hernandez SM, Balsamo G, Barron H, Beasley JC, Brown JD, Cloherty E, Farid H, Gabriel M, Groves B, Hamer S, Hill J, Lewis M, McManners K, Nemeth N, Oesterle P, Ortiz S, Peshock L, Schnellbacher R et al (2019) Prevalence, distribution, and diversity of cryptic piroplasm infections in raccoons from selected areas of the United States and Canada. Int J Parasitol Parasites Wildl 9:224–233. https://doi.org/10.1016/j.ijppaw.2019.05.007

Gebrekidan H, Perera PK, Ghafar A, Abbas T, Gasser RB, Jabbar A (2020) An appraisal of oriental theileriosis and the *Theileria orientalis* complex, with an emphasis on diagnosis and genetic characterisation. Parasitol Res 119:11–22. https://doi.org/10.1007/s00436-019-06557-7

George JE, Pound JM, Davey RB (2004) Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129(Suppl):S353–S366. https://doi.org/10.1017/s0031182003004682

Gharbi M, Darghouth MA, Elati K, Al-Hosary AAT, Ayadi O, Salih DA, El Hussein AM, Mhadhbi M, Khamassi Khbou M, Hassan SM, Obara I, Ahmed LS, Ahmed J (2020) Current status of tropical theileriosis in Northern Africa: a review of recent epidemiological investigations and implications for control. Transbound Emerg Dis 67(Suppl 1):8–25. https://doi.org/10.1111/tbed.13312

Glenn BL, Kocan AA, Blouin EF (1983) Cytauxzoonosis in bobcats. J Am Vet Med Assoc 183:1155–1158

Gonçalves LR, Paludo G, Bisol TB, Perles L, de Oliveira LB, de Oliveira CM, da Silva TMV, Nantes WAG, Duarte MA, Santos FM, de Oliveira Porfírio GE, Hirano LQL, Herrera HM, Barros-Battesti DM, Machado RZ, André MR (2021) Molecular detection of piroplasmids in synanthropic rodents, marsupials, and associated ticks from Brazil, with phylogenetic inference of a putative novel *Babesia* sp. from white-eared opossum (*Didelphis albiventris*). Parasitol Res 120:3537–3546. https://doi.org/10.1007/s00436-021-07284-8

Grause JF, Ueti MW, Nelson JT, Knowles DP, Kappmeyer LS, Bunn TO (2013) Efficacy of imidocarb dipropionate in eliminating *Theileria equi* from experimentally infected horses. Vet J 196:541–546. https://doi.org/10.1016/j.tvjl.2012.10.025

Gray A, Capewell P, Loney C, Katzer F, Shiels BR, Weir W (2019) Sheep as host species for zoonotic Babesia venatorum, United Kingdom. Emerg Infect Dis 25:2257–2260. https://doi.org/10.3201/eid2512

Gray JS, Estrada-Peña A, Zintl A (2019) Vectors of babesiosis. Annu Rev Entomol 64:149–165. https://doi.org/10.1146/annurev-ento-011118-111932

Greay TL, Zahedi A, Krige AS, Owens JM, Rees RL, Ryan UM, Oskam CL, Irwin PJ (2018) Endemic, exotic and novel apicomplexan parasites detected during a national study of ticks from companion animals in Australia. Parasit Vectors 11:197. https://doi.org/10.1186/s13071-018-2775-y

Greay TL, Zahedi A, Krige AS, Owens JM, Rees RL, Ryan UM et al (2019) Response to the letter to the editor by Harris. Parasit Vectors 12:178. https://doi.org/10.1186/s13071-019-3439-2

Guan G, Korhonen PK, Young ND, Koehler AV, Wang T, Li Y, Liu Z, Luo J, Yin H, Gasser RB (2016) Genomic resources for a unique, low-virulence *Babesia* taxon from China. Parasit Vectors 9:564. https://doi.org/10.1186/s13071-016-1846-1

Guan G, Ma M, Moreau E, Liu J, Lu B, Bai Q, Luo J, Jorgensen W, Chauvin A, Yin H (2009) A new ovine *Babesia* species transmitted by *Hyalomma anatolicum anatolicum*. Exp Parasitol 122:261–267

Gubbels JM, de Vos AP, van der Weide M, Viseras J, Schouls LM, de Vries E, Jongejan F (1999) Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. J Clin Microbiol 37:1782–1789. https://doi.org/10.1128/JCM.37.6.1782-1789.1999

Guo S, Yuan Z, Wu G, Wang W, Ma D, Du H (2002) Epidemiology of ovine theileriosis in Ganan region, Gansu Province, China. Parasitol Res 88(13 Suppl 1):S36–S37. https://doi.org/10.1007/s00436-001-0568-1

Hall CM, Busch JD, Scoles GA, Palma-Cagle KA, Ueti MW, Kappmeyer LS, Wagner DM (2013) Genetic characterization of *Theileria equi* infecting horses in North America: evidence for a limited source of U.S. introductions. Parasit Vectors 6(35). https://doi.org/10.1186/1756-3305-6-35

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape split by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Hashemi-Fesharki R, Uilenberg G (1981) *Babesia crassa* n.sp. (Sporozoa, Babesiidae) of domestic sheep in Iran. Vet Q 3:1–8. https://doi.org/10.1080/01652176.1981.9693787

Hassanin A, Delsuc F, Ropiquet A, Hammer C, Jansen van Vuuren B, Matthee C, Ruiz-Garcia M, Catzeflis F, Areskoug V, Nguyen TT, Couloux A (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. C R Biol 335:32–50. https://doi.org/10.1016/j.crvi.2011.11.002

He L, Liu Q, Yao B, Zhou Y, Hu M, Fang R, Zhao J (2017) A historical overview of research on *Babesia orientalis*, a protozoan parasite infecting water buffalo. Front Microbiol 8:1323. https://doi.org/10.3389/fmicb.2017.01323

Henker LC, Lorenzett MP, Fagundes-Moreira R, Dalto AGC, Sonne L, Driemeier D, Soares JF, Pavarini SP (2020) Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* sp. infections in southern Brazil. Ticks Tick Borne Dis 11:101443. https://doi.org/10.1016/j.ttbdis.2020.101443

Henning MW (1956) Animal diseases in South Africa, 3rd edn. Central News Agency, Ltd., South Africa

Hooshmand-Rad P, Hawa NJ (1973a) Transmission of *Theileria hirci* in sheep by Hyalomma anatolicum anatolicum. Trop Anim Health Prod 5:100–109

Hooshmand-Rad P, Hawa NJ (1973b) Malignant theileriosis of sheep and goats. Trop Anim Health Prod 5:97–102

Hrazdilová K, Rybářová M, Široký P, Votýpka J, Zintl A, Burgess H, Steinbauer V, Žákovčík V, Modrý D (2020) Diversity of *Babesia* spp. in cervid ungulates based on the 18S rDNA and cytochrome c oxidase subunit I phylogenies. Infect Genet Evol 77:104060. https://doi.org/10.1016/j.meegid.2019.104060

Hurtado A, Barandika JF, Oporto B, Minguijón E, Povedano I, García-Pérez AL (2015) Risks of suffering tick-borne diseases in sheep translocated to a tick infested area: a laboratory approach for the investigation of an outbreak. Ticks Tick Borne Dis 6:31–37. https://doi.org/10.1016/j.ttbdis.2014.09.001

Hutcheon D (1890) Biliary fever or jaundice in the horse. Agric J Cape Good Hope 2:361–362

Ikeda P, Menezes TR, Torres JM, de Oliveira CE, Lourenço EC, Herrera HM, Machado RZ, André MR (2021) First molecular detection of piroplasmids in non-hematophagous bats from Brazil, with evidence of putative novel species. Parasitol Res 120:301–310. https://doi.org/10.1007/s00436-020-06985-w

Inácio EL, Pérez-Macchi S, Alabi A, Bittencourt P, Müller A (2019) Prevalence and molecular characterization of piroplasmids in domestic dogs from Paraguay. Ticks Tick Borne Dis 10:321–327. https://doi.org/10.1016/j.ttbdis.2018.11.009

Inci A, Yildirim A, Duzlu O, Doganay M, Aksoy S (2016) Tick-borne diseases in Turkey: a review based on one health perspective. PLoS Negl Trop Dis 10:e0005021. https://doi.org/10.1371/journal.pntd.0005021

Inokuma H, Yoshizaki Y, Shimada Y, Sakata Y, Okuda M, Onishi T (2003) Epidemiological survey of *Babesia* species in Japan performed with specimens from ticks collected from dogs and detection of new *Babesia* DNA closely related to *Babesia odocoilei* and *Babesia divergens* DNA. J Clin Microbiol 41:3494–3498. https://doi.org/10.1128/JCM.41.8.3494-3498.2003

Jalovecka M, Hajdusek O, Sojka D, Kopacek P, Malandrin L (2018) The complexity of piroplasms life cycles. Front Cell Infect Microbiol 8:248. https://doi.org/10.3389/fcimb.2018.00248

Jalovecka M, Sojka D, Ascencio M, Schnittger L (2019) *Babesia* life cycle—when phylogeny meets biology. Trends Parasitol 35:356–368. https://doi.org/10.1016/j.pt.2019.01.007

Jenkins C, Micallef M, Alex SM, Collins D, Djordjevic SP, Bogema DR (2015) Temporal dynamics and subpopulation analysis of *Theileria orientalis* genotypes in cattle. Infect Genet Evol 32:199–207. https://doi.org/10.1016/j.meegid.2015.03.017

Jiang Y, Xie M, Chen W, Talbot R, Maddox JF, Faraut T et al (2014) The sheep genome illuminates biology of the rumen and lipid metabolism. Science 344:1168–1173. https://doi.org/10.1126/science.1252806

Jinnai M, Kawabuchi-Kurata T, Tsuji M, Nakajima R, Hirata H, Fujisawa K, Shiraki H, Asakawa M, Nasuno T, Ishihara C (2010) Molecular evidence of the multiple genotype infection of a wild Hokkaido brown bear (Ursus arctos yesoensis) by *Babesia* sp. UR1. Vet Parasitol 173:128–133. https://doi.org/10.1016/j.vetpar.2010.06.018

Jonsson NN, Bock RE, Jorgensen WK (2008) Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses, and the effects of differing intensity of tick control in Australia. Vet Parasitol 155:1–9. https://doi.org/10.1016/j.vetpar.2008.03.022

Jouglin M, Fernández-de-Mera IG, de la Cotte N, Ruiz-Fons F, Gortázar C, Moreau E, Bastian S, de la Fuente J, Malandrin L (2014) Isolation and characterization of *Babesia pecorum* sp. nov. from farmed red deer (*Cervus elaphus*). Vet Res 45:78. https://doi.org/10.1186/s13567-014-0078-7

Kakoma I, Mehlhorn H (1994) Babesia of domestic ruminants. In: In: Kreier, J.P., Baker, J.R. (ed) Parasitic protozoa, vol 7. Academic Press, New York, pp 141–216

Kamau J, de Vos AJ, Playford M, Salim B, Kinyanjui P, Sugimoto C (2011) Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. Parasit Vectors 4:22. https://doi.org/10.1186/1756-3305-4-22

Kappmeyer LS, Thiagarajan M, Herndon DR, Ramsay JD, Caler E, Djikeng A, Gillespie JJ, Lau AO, Roalson EH, Silva JC, Silva MG, Suarez CE, Ueti MW, Nene VM, Mealey RH, Knowles DP, Brayton KA (2012) Comparative genomic analysis and phylogenetic position of *Theileria equi*. BMC Genomics 13:603. https://doi.org/10.1186/1471-2164-13-603

Ketz-Riley CJ, Reichard MV, Van den Bussche RA, Hoover JP, Meinkoth J, Kocan AA (2003) An intraerythrocytic small piroplasm in wild-caught Pallas's cats (*Otocolobus manul*) from Mongolia. J Wildl Dis 39:424–430. https://doi.org/10.7589/0090-3558-39.2.424

Kiara H, Steinaa L, Vishvanath N, Svitek N (2018) *Theileria* in ruminants. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 215–239

Kier AB (1979) In: The etiology and pathogenesis of feline cytauxzoonosis. PhD dissertation, University of Missouri, Columbia

Kier AB, Wightman SR, Wagner JE (1982) Interspecies transmission of *Cytauxzoon felis*. Am J Vet Res 43(1):102–105

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581

Kjemtrup AM, Wainwright K, Miller M, Penzhorn BL, Carreno RA (2006) *Babesia conradae*, sp. nov., a small canine *Babesia* identified in California. Vet Parasitol 138:103–111. https://doi.org/10.1016/j.vetpar.2006.01.044

Knowles DP, Kappmeyer LS, Haney D, Herndon DR, Fry LM, Munro JB, Sears K, Ueti MW, Wise LN, Silva M, Schneider DA, Grause J, White SN, Tretina K, Bishop RP, Odongo DO, Pelzel-McCluskey AM, Scoles GA, Mealey RH, Silva JC (2018) Discovery of a novel species, *Theileria haneyi* n. sp., infective to equids, highlights exceptional genomic diversity within the genus *Theileria*: implications for apicomplexan parasite surveillance. Int J Parasitol 48:679–690. https://doi.org/10.1016/j.ijpara.2018.03.010

Kocan AA, Kocan KM, Blouin EF, Mukolwe SW (1992) A redescription of schizogony of *Cytauxzoon felis* in the domestic cat. Ann N Y Acad Sci 653:161–167. https://doi.org/10.1111/j.1749-6632.1992.tb19639.x

Kumar B, Manjunathachar HV, Ghosh S (2020) A review on *Hyalomma* species infestations on human and animals and progress on management strategies. Heliyon. 6:e05675. https://doi.org/10.1016/j.heliyon.2020.e05675

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Lack JB, Reichard MV, Van Den Bussche RA (2012) Phylogeny and evolution of the Piroplasmida as inferred from 18S rRNA sequences. Int J Parasitol 42:353–363. https://doi.org/10.1016/j.ijpara.2012.02.005

Laus F, Spaterna A, Faillace V, Veronesi F, Ravagnan S, Beribé F, Cerquetella M, Meligrana M, Tesei B (2015) Clinical investigation on *Theileria equi* and *Babesia caballi* infections in Italian donkeys. BMC Vet Res 11:100. https://doi.org/10.1186/s12917-015-0411-z

Laveran A (1901) Contribution a' l'etude du Piroplasma equi. Societe de Biologie et de ses Filiales 53:385–388

Leclaire S, Menard S, Berry A (2015) Molecular characterization of *Babesia* and *Cytauxzoon* species in wild South-African meerkats. Parasitology 142:543–548. https://doi.org/10.1017/S0031182014001504

Leemans I, Brown D, Hooshmand-Rad P, Kirvar E, Uggla A (1999) Infectivity and cross-immunity studies of *Theileria lestoquardi* and *Theileria annulata* in sheep and cattle: I. In vivo responses. Vet Parasitol 82:179–192. https://doi.org/10.1016/s0304-4017(99)00013-8

Leemans I, Hooshmand-Rad P, Brown CG, Kirvar E, Wilkie G, Uggla A (1998) In vitro infectivity and in vivo cross-protectivity of *Theileria lestoquardi* and *T. annulata* in sheep and cattle. Ann N Y Acad Sci 849:408–411. https://doi.org/10.1111/j.1749-6632.1998.tb11083.x

Leemans I, Hooshmand-Rad P, Uggla A (1997) The indirect fluorescent antibody test based on schizont antigen for study of the sheep parasite *Theileria lestoquardi*. Vet Parasitol 69:9–18. https://doi.org/10.1016/s0304-4017(96)01098-9

Levine ND (1985) Veterinary parasitology. Lowa State University Press, Ames, pp 291–312

Levine ND, Corliss JO, Cox FE, Deroux G, Grain J, Honigberg BM, Leedale GF, Loeblich AR 3rd, Lom J, Lynn D, Merinfeld EG, Page FC, Poljansky G, Sprague V, Vavra J, Wallace FG (1980) A newly revised classification of the protozoa. J Protozool 27:37–58. https://doi.org/10.1111/j.1550-7408.1980.tb04228.x

Lewis KM, Cohn LA, Downey ME, Whitney MS, Birkenheuer AJ (2012) Evaluation of *Cytauxzoon felis* infection status in captive-born wild felids housed in an area endemic for the pathogen. J Am Vet Med Assoc 241:1088–1092. https://doi.org/10.2460/javma.241.8.1088

Lewis D, Herbert IV (1980) A large Babesia of sheep from North Wales. Vet Rec 107:352

Lewis D, Holman MR, Purnell RE, Young ER, Herbert IV, Bevan WJ (1981) Investigations on *Babesia motasi* isolated from Wales. Res Vet Sci 31:239–243

Li Y, Chen Z, Liu Z, Liu J, Yang J, Li Q, Li Y, Cen S, Guan G, Ren Q, Luo J, Yin H (2014) Molecular identification of *Theileria* parasites of northwestern Chinese Cervidae. Parasit Vectors 7:225. https://doi.org/10.1186/1756-3305-7-225

Li K, Shahzad M, Zhang H, Jiang X, Mehmood K, Zhao X, Li J (2018) Socio-economic burden of parasitic infections in yaks from 1984 to 2017 on Qinghai Tibetan Plateau of China—a review. Acta Trop 183:103–109. https://doi.org/10.1016/j.actatropica.2018.04.011

Li LH, Wang JZ, Zhu D, Li XS, Lu Y, Yin SQ, Li SG, Zhang Y, Zhou XN (2020) Detection of novel piroplasmid species and *Babesia microti* and *Theileria orientalis* genotypes in hard ticks from Tengchong County, Southwest China. Parasitol Res 119:1259–1269. https://doi.org/10.1007/s00436-020-06622-6

Librado P, Orlando L (2021) Genomics and the evolutionary history of equids. Annu Rev Anim Biosci 9:81–101. https://doi.org/10.1146/annurev-animal-061220-023118

Liu J, Guan G, Li Y, Liu A, Luo J, Yin H (2017) A molecular survey of *Babesia* species and detection of a new *Babesia* species by DNA related to *B. venatorum* from white yaks in Tianzhu, China. Front Microbiol 8:419. https://doi.org/10.3389/fmicb.2017.00419

Liu J, Yang J, Guan G, Liu A, Wang B, Luo J, Yin H (2016) Molecular detection and identification of piroplasms in sika deer (*Cervus nippon*) from Jilin Province, China. Parasit Vectors 9:156. https://doi.org/10.1186/s13071-016-1435-3 Erratum in: Parasit Vectors. 2016;9:188

Liu AH, Yin H, Guan GQ, Schnittger L, Liu ZJ, Ma ML, Dang ZS, Liu JL, Ren QY, Bai Q, Ahmed JS, Luo JX (2007) At least two genetically distinct large *Babesia* species infective to sheep and goats in China. Vet Parasitol 147:246–251. https://doi.org/10.1016/j.vetpar.2007.03.032

Liu Z, Zhao J, Ma L, Yao B (1997) *Babesia orientalis* sp. nov. parasitized in buffalo *Bubalus bubalis* in China (Piroplasmida: Babesiidae). Acta Vet Zootech Sin 28:89

Loh SM, Egan S, Gillett A, Banks PB, Ryan UM, Irwin PJ, Oskam CL (2018) Molecular surveillance of piroplasms in ticks from small and medium-sized urban and peri-urban mammals in Australia. Int J Parasitol Parasites Wildl 7:197–203. https://doi.org/10.1016/j.ijppaw.2018.05.005

Loretti AP, Barros SS (2005) Hemorrhagic disease in dogs infected with an unclassified intraendothelial piroplasm in southern Brazil. Vet Parasitol 134:193–213. https://doi.org/10.1016/j.vetpar.2005.07.011

Luo J, Yin H (1997) Theileriosis of sheep and goats in China. Trop Anim Health Prod 29:8S–10S. https://doi.org/10.1007/BF02632907

Luo J, Yin H, Guan G, Zhang Q, Lu W (2002) Description of a new *Babesia* sp. infective for cattle in China. Parasitol Res 88:S13–S15. https://doi.org/10.1007/s00436-001-0562-7

Luo J, Yin H, Liu Z, Yang D, Guan G, Liu A, Ma M, Dang S, Lu B, Sun C, Bai Q, Lu W, Chen P (2005) Molecular phylogenetic studies on an unnamed bovine *Babesia* sp. based on small subunit ribosomal RNA gene sequences. Vet Parasitol 133:1–6. https://doi.org/10.1016/j.vetpar.2005.02.014

Maboko BB, Sibeko-Matjila KP, Pierneef R, Chan WY, Josemans A, Marumo RD, Mbizeni S, Latif AA, Mans BJ (2021) South African buffalo-derived *Theileria parva* is distinct from other buffalo and cattle-derived *T. parva*. Front Genet 12:666096. https://doi.org/10.3389/fgene.2021.666096

Malandrin L (2021) *Babesia bennetti*: a chimeric sequence for a true parasite? Ticks Tick Borne Dis 13:101863. https://doi.org/10.1016/j.ttbdis.2021.101863

Malangmei L, Ajith Kumar KG, Nandini A, Bora CAF, Varghese A, Amrutha BM, Kurbet PS, Pradeep RK, Nimisha M, Deepa CK, John L, Ravindran R (2021) Molecular characterization of hemoparasites and hemoplasmas infecting domestic cats of southern India. Front Vet Sci 7:597598. https://doi.org/10.3389/fvets.2020.597598

Malheiros J, Costa MM, do Amaral RB, de Sousa KCM, André MR, Machado RZ, Vieira MIB (2016) Identification of vector-borne pathogens in dogs and cats from Southern Brazil. Ticks Tick Borne Dis 7:893–900. https://doi.org/10.1016/j.ttbdis.2016.04.007

Man SQ, Qiao K, Cui J, Feng M, Fu YF, Cheng XJ (2016) A case of human infection with a novel *Babesia* species in China. Infect Dis Poverty 5:28. https://doi.org/10.1186/s40249-016-0121-1

Manna G, Cersini A, Nardini R, Bartolomé Del Pino LE, Antognetti V, Zini M, Conti R, Lorenzetti R, Veneziano V, Autorino GL, Scicluna MT (2018) Genetic diversity of *Theileria equi* and *Babesia caballi* infecting horses of Central-Southern Italy and preliminary results of its correlation with clinical and serological status. Ticks Tick Borne Dis 9:1212–1220. https://doi.org/10.1016/j.ttbdis.2018.05.005

Mans BJ, Pienaar R, Latif AA (2015) A review of *Theileria* diagnostics and epidemiology. Int J Parasitol Parasites Wildl 4:104–118. https://doi.org/10.1016/j.ijppaw.2014.12.006

Mans BJ, Pienaar R, Latif AA, Potgieter FT (2011) Diversity in the 18S SSU rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitology 138:766–779. https://doi.org/10.1017/S0031182011000187

Maritz-Olivier C, Stutzer C, Jongejan F, Neitz AW, Gaspar AR (2007) Tick anti-hemostatics: targets for future vaccines and therapeutics. Trends Parasitol 23:397–407. https://doi.org/10.1016/j.pt.2007.07.005

Martínez-García G, Santamaría-Espinosa RM, Lira-Amaya JJ, Figueroa JV (2021) Challenges in tick-borne pathogen detection: the case for Babesia spp. identification in the tick vector. Pathogens. 10:92. https://doi.org/10.3390/pathogens10020092

Maya-Delgado A, Madder M, Benítez-Ortíz W, Saegerman C, Berkvens D, Ron-Garrido L (2020) Molecular screening of cattle ticks, tick-borne pathogens and amitraz resistance in ticks of Santo Domingo de los Tsáchilas province in Ecuador. Ticks Tick Borne Dis 11:101492. https://doi.org/10.1016/j.ttbdis.2020.101492

McIntosh MT, Srivastava R, Vaidya AB (1998) Divergent evolutionary constraints on mitochondrial and nuclear genomes of malaria parasites. Mol Biochem Parasitol 95(1):69–80. https://doi.org/10.1016/s0166-6851(98)00093-0

Mehlhorn H, Schein E (1998) Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1998. Parasitol Res 84:467–475. https://doi.org/10.1007/s004360050431

Mehlhorn H, Schein E, Ahmed JS (1994) *Theileria*. In: Kreier J P. Baker JR. Parasitic protozoa. Academic Press, New York, pp 217–304

Meli ML, Cattori V, Martínez F, López G, Vargas A, Simón MA, Zorrilla I, Muñoz A, Palomares F, López-Bao JV, Pastor J, Tandon R, Willi B, Hofmann-Lehmann R, Lutz H (2009) Feline leukemia virus and other pathogens as important threats to the survival of the critically endangered Iberian lynx (*Lynx pardinus*). PLoS One 4:e4744. https://doi.org/10.1371/journal.pone.0004744

Millán J, Rodriguéz N, Pérez de la Lastra J, Mangold A, de la Fuente J (2007) Prevalence of infection and 18S rRNA gene sequences of *Cytauxzoon* species in Iberian lynx (*Lynx pardinus*) in Spain. Parasitology 134:995–1001. https://doi.org/10.1017/S003118200700248X

Morrison WI, Connelley T, Hemmink JD, MacHugh ND (2015) Understanding the basis of parasite strain-restricted immunity to *Theileria parva*. Annu Rev Anim Biosci 3:397–418. https://doi.org/10.1146/annurev-animal-022513-114152

Morrison WI, Hemmink JD, Toye PG (2020) Theileria parva: a parasite of African buffalo, which has adapted to infect and undergo transmission in cattle. Int J Parasitol 50:403–412. https://doi.org/10.1016/j.ijpara.2019.12.006

Morrison WI, McKeever DJ (2006) Current status of vaccine development against *Theileria* parasites. Parasitology 133:S169–S187. https://doi.org/10.1017/S0031182006001867

Mosqueda J, Olvera-Ramirez A, Aguilar-Tipacamu G, Canto GJ (2012) Current advances in detection and treatment of babesiosis. Curr Med Chem 19:1504–1518. https://doi.org/10.2174/092986712799828355

Moubri K, Kleuskens J, Van de Crommert J, Scholtes N, Van Kasteren T, Delbecq S, Carcy B, Précigout E, Gorenflot A, Schetters T (2018) Discovery of a recombinant *Babesia canis* supernatant antigen that protects dogs against virulent challenge infection. Vet Parasitol 249:21–29. https://doi.org/10.1016/j.vetpar.2017.11.002

Moustafa MAM, Sasaki A, Shimozuru M, Nakao R, Sashika M, Yamazaki K, Koike S, Tanaka J, Tamatani H, Yamanaka M, Ishinazaka T, Tsubota T (2020) Molecular detection of apicomplexan protozoa in Hokkaido brown bears (*Ursus arctos yesoensis*) and Japanese black bears (Ursus thibetanus japonicus). Parasitol Res 119:3739–3753. https://doi.org/10.1007/s00436-020-06873-3

Mshelia PW, Kappmeyer L, Johnson WC, Kudi CA, Oluyinka OO, Balogun EO, Richard EE, Onoja E, Sears KP, Ueti MW (2020) Molecular detection of *Theileria* species and *Babesia caballi* from horses in Nigeria. Parasitol Res 119:2955–2963. https://doi.org/10.1007/s00436-020-06797-y

Munkhjargal T, Sivakumar T, Battsetseg B, Nyamjargal T, Aboulaila M, Purevtseren B, Bayarsaikhan D, Byambaa B, Terkawi MA, Yokoyama N, Igarashi I (2013) Prevalence and genetic diversity of equine piroplasms in Tov province. Mongolia Infect Genet Evol 16:178–185. https://doi.org/10.1016/j.meegid.2013.02.005

Muñoz-Gómez SA, Durnin K, Eme L, Paight C, Lane CE, Saffo MB, Slamovits CH (2019) Nephromyces represents a diverse and novel lineage of the Apicomplexa that has retained apicoplasts. Genome Biol Evol 11:2727–2740. https://doi.org/10.1093/gbe/evz155

Musoke A, Rowlands J, Nene V, Nyanjui J, Katende J, Spooner P, Mwaura S, Odongo D, Nkonge C, Mbogo S, Bishop R, Morzaria S (2005) Subunit vaccine based on the p67 major surface protein of *Theileria parva* sporozoites reduces severity of infection derived from field tick challenge. Vaccine 23:3084–3095. https://doi.org/10.1016/j.vaccine.2004.09.039

Nagore D, García-Sanmartín J, García-Pérez AL, Juste RA, Hurtado A (2004a) Detection and identification of equine *Theileria* and *Babesia* species by reverse line blotting: epidemiological survey and phylogenetic analysis. Vet Parasitol 123:41–54. https://doi.org/10.1016/j.vetpar.2004.04.010

Nagore D, García-Sanmartín J, García-Pérez AL, Juste RA, Hurtado A (2004b) Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in a sheep population from Northern Spain. Int J Parasitol 34:1059–1067. https://doi.org/10.1016/j.ijpara.2004.05.008

Ndawula C Jr, Tabor AE (2020) Cocktail anti-tick vaccines: the unforeseen constraints and approaches toward enhanced efficacies. Vaccines (Basel) 8:457. https://doi.org/10.3390/vaccines8030457

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

Nentwig A, Meli ML, Schrack J, Reichler IM, Riond B, Gloor C, Howard J, Hofmann-Lehmann R, Willi B (2018) First report of *Cytauxzoon* sp. infection in domestic cats in Switzerland: natural and transfusion-transmitted infections. Parasit Vectors:11:292. https://doi.org/10.1186/s13071-018-2728-5

Nicaretta JE, Zapa DMB, Couto LFM, Heller LM, Cavalcante ASA, Cruvinel LB, Melo Júnior RD, Ferreira LL, Nascimento RMD, Soares VE, Borges LMF, Monteiro CMO, Lopes WDZ (2021) *Rhipicephalus microplus* seasonal dynamic in a Cerrado biome, Brazil: an update data considering the global warming. Vet Parasitol 296:109506. https://doi.org/10.1016/j.vetpar.2021.109506

Niu Q, Liu Z, Yang J, Gao S, Pan Y, Guan G, Luo J, Yin H (2017) Genetic characterization and molecular survey of *Babesia* sp. Xinjiang infection in small ruminants and ixodid ticks in China. Infect Genet Evol 49:330–335. https://doi.org/10.1016/j.meegid.2017.01.025

Niu Q, Liu Z, Yang J, Yu P, Pan Y, Zhai B, Luo J, Yin H (2016) Genetic diversity and molecular characterization of *Babesia motasi*-like in small ruminants and ixodid ticks from China. Infect Genet Evol 41:8–15. https://doi.org/10.1016/j.meegid.2016.03.007

Noaman V, Ghadimipour R, Nabavi R (2021) First report of *Babesia occultans* in two symptomatic cows in Iran. Parasitol Res 120:1915–1919. https://doi.org/10.1007/s00436-021-07119-6

Nunn JA (1894) Specific fevers of malarial origin in equines. Vet J 39:402–405

Nuttall GHF, Stickland C (1912) On the occurrence of two species of parasites in equine piroplasmosis or biliary fever. Parasitology 12:65

Nuttall GHF, Strickland C (1910) Die parasiten des pferdepiroplasmose resp. der "Bilary fever". Centralblatt fur Bakteriologie, Parasitenkunde und Infektionskrankheiten Abteilung I 56:524–525

OIE (2021) Manual of diagnostic tests and vaccines for terrestrial animals https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/, Equine piroplasmosis. Chapter 3.6.8

Onyiche TE, Suganuma K, Igarashi I, Yokoyama N, Xuan X, Thekisoe O (2019) A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. Int J Environ Res Public Health 16:1736. https://doi.org/10.3390/ijerph16101736

Otgonsuren D, Sivakumar T, Amgalanbaatar T, Enkhtaivan B, Narantsatsral S, Tuvshintulga B, Zoljargal M, Munkhgerel D, Davkharbayar B, Baatarjargal P, Davaasuren B, Myagmarsuren P, Battsetseg B, Battur B, Yokoyama N (2020) Molecular epidemiological survey of *Babesia bovis, Babesia bigemina,* and *Babesia* sp. Mymensingh infections in Mongolian cattle. Parasitol Int 77:102107. https://doi.org/10.1016/j.parint.2020.102107

Ouedraogo AS, Zannou OM, Biguezoton AS, Yao KP, Belem AMG, Farougou S, Oosthuizen M, Saegerman C, Lempereur L (2021) Cross border transhumance involvement in ticks and tick-borne pathogens dissemination and first evidence of *Anaplasma centrale* in Burkina Faso. Ticks Tick Borne Dis 12:101781. https://doi.org/10.1016/j.ttbdis.2021.101781

Panait LC, Mihalca AD, Modrý D, Juránková J, Ionică AM, Deak G, Gherman CM, Heddergott M, Hodžić A, Veronesi F, Reichard M, Zieman EA, Nielsen CK, Jiménez-Ruiz FA, Hrazdilová K (2021) Three new species of *Cytauxzoon* in European wild felids. Vet Parasitol 290:109344. https://doi.org/10.1016/j.vetpar.2021.109344

Panait LC, Stock G, Globokar M, Balzer J, Groth B, Mihalca AD, Pantchev N (2020) First report of *Cytauxzoon* sp. infection in Germany: organism description and molecular confirmation in a domestic cat. Parasitol Res 119:3005–3011. https://doi.org/10.1007/s00436-020-06811-3

Panicker VP, Sreedharannair AK, Narayanan A, George S, Hameed SV (2020) Molecular identification of a novel species, *Babesia panickeri* sp. nov., from a naturally infected domestic cat of India and its comparison with canine *Babesia* isolates. Acta Parasitol 65:913–918. https://doi.org/10.2478/s11686-020-00228-9

Paparini A, Macgregor J, Ryan UM, Irwin PJ (2015) First molecular characterization of *Theileria ornithorhynchi* Mackerras, 1959: yet another challenge to the systematics of the piroplasms. Protist. 166:609–620. https://doi.org/10.1016/j.protis.2015.10.001

Paparini A, McInnes LM, Di Placido D, Mackereth G, Tompkins DM, Clough R, Ryan UM, Irwin PJ (2014) Piroplasms of New Zealand seabirds. Parasitol Res 113:4407–4414. https://doi.org/10.1007/s00436-014-4118-z

Paparini A, Ryan UM, Warren K, McInnes LM, de Tores P, Irwin PJ (2012) Identification of novel *Babesia* and *Theileria* genotypes in the endangered marsupials, the woylie (*Bettongia penicillata ogilbyi*) and boodie (*Bettongia lesueur*). Exp Parasitol 131:25–30. https://doi.org/10.1016/j.exppara.2012.02.021

Park Y, West RA, Pathmendra P, Favier B, Stoeger T, Capes-Davis A, Cabanac G, Labbé C, Byrne JA (2021) Human gene function publications that describe wrongly identified nucleotide sequence reagents are unacceptably frequent within the genetics literature. https://doi.org/10.1101/2021.07.29.453321

Patton WS (1910) Preliminary report on a new piroplasm (*Piroplasma gibsoni* sp. nov.) found in the blood of the hounds of the Madras Hunt and subsequently discovered in the blood of the jackal Canis aureus. Bull Soc Pathol Exot 3:274–280

Pedrassani D, Biolchi J, Gonçalves LR, Mendes NS, Zanatto DCS, Calchi AC, Machado RZ, André MR (2019) Molecular detection of vector-borne agents in cats in Southern Brazil. Rev Bras Parasitol Vet 28:632–643. https://doi.org/10.1590/S1984-29612019077

Penzhorn BL (2011) Why is Southern African canine babesiosis so virulent? An evolutionary perspective. Parasit Vectors 4:51. https://doi.org/10.1186/1756-3305-4-51

Penzhorn BL, Kjemtrup AM, López-Rebollar LM, Conrad PA (2001) *Babesia leo* n. sp. from lions in the Kruger National Park, South Africa, and its relation to other small piroplasms. J Parasitol 87:681–685. https://doi.org/10.1645/0022-3395(2001)087[0681:BLNSFL]2.0.CO;2

Penzhorn BL, Oosthuizen MC (2020) *Babesia* species of domestic cats: molecular characterization has opened pandora's box. Front Vet Sci 7:134. https://doi.org/10.3389/fvets.2020.00134

Penzhorn BL, Vorster I, Harrison-White RF, Oosthuizen MC (2017) Black-backed jackals (*Canis mesomelas*) are natural hosts of *Babesia rossi*, the virulent causative agent of canine babesiosis in sub-Saharan Africa. Parasit Vectors 10:124. https://doi.org/10.1186/s13071-017-2057-0

Pfister K, Armstrong R (2016) Systemically and cutaneously distributed ectoparasiticides: a review of the efficacy against ticks and fleas on dogs. Parasit Vectors 9:436. https://doi.org/10.1186/s13071-016-1719-7

Phipps LP, Hernández-Triana LM, Goharriz H, Welchman D, Johnson N (2016) Detection of Theileria luwenshuni in sheep from Great Britain. Parasit Vectors 9:203. https://doi.org/10.1186/s13071-016-1486-5

Pienaar R, Latif AA, Mans BJ (2018) Investigations into the host specificity of *Theileria taurotragi*. Vet Parasitol 254:30–35. https://doi.org/10.1016/j.vetpar.2018.03.003

Preston PM (2001) Theileriosis. In: Service MW (ed) The encyclopedia of arthropod-transmitted infections. CAB International, Wallingford, pp 487–504

Qablan MA, Oborník M, Petrželková KJ, Sloboda M, Shudiefat MF, Hořín P, Lukeš J, Modrý D (2013) Infections by Babesia caballi and Theileria equi in Jordanian equids: epidemiology and genetic diversity. Parasitology. 140:1096–1103. https://doi.org/10.1017/S0031182013000486

Qablan MA, Sloboda M, Jirků M, Oborník M, Dwairi S, Amr ZS, Hořín P, Lukeš J, Modrý D (2012) Quest for the piroplasms in camels: identification of Theileria equi and Babesia caballi in Jordanian dromedaries by PCR. Vet Parasitol 186:456–460. https://doi.org/10.1016/j.vetpar.2011.11.070

Ranjbar-Bahadori S, Eckert B, Omidian Z, Shirazi NS, Shayan P (2012) *Babesia ovis* as the main causative agent of sheep babesiosis in Iran. Parasitol Res 110:1531–1536. https://doi.org/10.1007/s00436-011-2658-z

Razmi GR, Hosseini M, Aslani MR (2003) Identification of tick vectors of ovine theileriosis in an endemic region of Iran. Vet Parasitol 116:1–6. https://doi.org/10.1016/s0304-4017(03)00254-1

Reichard MV, Edwards AC, Meinkoth JH, Snider TA, Meinkoth KR, Heinz RE, Little SE (2010) Confirmation of *Amblyomma americanum* (Acari: Ixodidae) as a vector for Cytauxzoon *felis* (Piroplasmorida: Theileriidae) to domestic cats. J Med Entomol 47:890–896. https://doi.org/10.1603/me10013

Reichard M, Meinkoth J, Edwards A, Snider T, Kocan K, Blouin E, Little S (2009) Transmission of Cytauxzoon felis to a domestic cat by Amblyomma americanum. Vet Parasitol 161:110–115

Reichard MV, Van Den Bussche RA, Meinkoth JH, Hoover JP, Kocan AA (2005) A new species of *Cytauxzoon* from Pallas' cats caught in Mongolia and comments on the systematics and taxonomy of piroplasmids. J Parasitol 91:420–426. https://doi.org/10.1645/GE-384R

Rjeibi MR, Gharbi M, Mhadhbi M, Mabrouk W, Ayari B, Nasfi I, Jedidi M, Sassi L, Rekik M, Darghouth MA (2014) Prevalence of piroplasms in small ruminants in North-West Tunisia and the first genetic characterisation of *Babesia ovis* in Africa. Parasite 21:23. https://doi.org/10.1051/parasite/2014025

Rodriguez AE, Schnittger L, Tomazic ML, Florin-Christensen M (2013) Current and prospective tools for the control of cattle-infecting *Babesia* parasites. In: Castillo V, Harris R (eds) Protozoa: biology, classification and role in disease. Hauppauge, Nova, pp 1–44

Romero-Salas D, Mira A, Mosqueda J, García-Vázquez Z, Hidalgo-Ruiz M, Vela NA, de León AA, Florin-Christensen M, Schnittger L (2016) Molecular and serological detection of *Babesia bovis*- and *Babesia bigemina*-infection in bovines and water buffaloes raised jointly in an endemic field. Vet Parasitol 217:101–107. https://doi.org/10.1016/j.vetpar.2015.12.030

Rosa CT, Pazzi P, Nagel S, McClure V, Christie J, Troskie M et al (2014) Theileriosis in six dogs in South Africa and its potential clinical significance. J S Afr Vet Assoc 85:1114

Ros-García A, M'ghirbi Y, Hurtado A, Bouattour A (2013) Prevalence and genetic diversity of piroplasm species in horses and ticks from Tunisia. Infect Genet Evol 17:33–37. https://doi.org/10.1016/j.meegid.2013.03.038

Rothschild CM (2013) Equine piroplasmosis. J Equine Vet Sci 33:497–508. https://doi.org/10.1016/j.jevs.2013.03.189

Roy BC, Krücken J, Ahmed JS, Majumder S, Baumann MP, Clausen PH, Nijhof AM (2018) Molecular identification of tick-borne pathogens infecting cattle in Mymensingh district of Bangladesh reveals emerging species of *Anaplasma* and *Babesia*. Transbound Emerg Dis 65:e231–e242. https://doi.org/10.1111/tbed.12745

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Saleh MN, Allen KE, Lineberry MW, Little SE, Reichard MV (2021) Ticks infesting dogs and cats in North America: biology, geographic distribution, and pathogen transmission. Vet Parasitol 294:109392. https://doi.org/10.1016/j.vetpar.2021.109392

Salim B, Bakheit MA, Kamau J, Nakamura I, Sugimoto C (2010) Nucleotide sequence heterogeneity in the small subunit ribosomal RNA gene within Theileria equi from horses in Sudan. Parasitol Res 106:493–498. https://doi.org/10.1007/s00436-009-1691-7

Sant C, Allicock OM, d'Abadie R, Charles RA, Georges K (2019) Phylogenetic analysis of *Theileria equi* and *Babesia caballi* sequences from thoroughbred mares and foals in Trinidad. Parasitol Res 118:1171–1177

Sant C, d'Abadie R, Pargass I, Basu AK, Asgarali Z, Charles RA, Georges KC (2016) Prospective study investigating transplacental transmission of equine piroplasmosis in thoroughbred foals in Trinidad. Vet Parasitol 226:132–137. https://doi.org/10.1016/j.vetpar.2016.07.008

Schein H (1917) Equine piroplasmosis in South Annam, French Indo-China. Bulletin de la Société de Pathologie Exotique 10:871–873

Schein E (1988) Equine babesiosis. In: Ristic M (ed) Babesiosis of domestic animals and man. CRC press, Boca Raton, pp 198–208

Schein E, Rehbein G, Voigt WP, Zweygarth E (1981) *Babesia equi* (Laveran 1901) 1. Development in horses and in lymphocyte culture. Tropenmed Parasitol 32:223–227

Schetters T (2005) Vaccination against canine babesiosis. Trends Parasitol 21:179–184. https://doi.org/10.1016/j.pt.2005.02.006

Schetters TP, Kleuskens J, Carcy B, Gorenflot A, Vermeulen A (2007) Vaccination against large *Babesia* species from dogs. Parassitologia 49:13–17

Schnittger L, Florin-Christensen M (2018) Introduction into parasitic protozoa. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 1–10

Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA (2012) *Babesia*: a world emerging. Infect Genet Evol 12:1788–1809. https://doi.org/10.1016/j.meegid.2012.07.004

Schnittger L, Yin H, Gubbels MJ, Beyer D, Niemann S, Jongejan F, Ahmed JS (2003) Phylogeny of sheep and goat *Theileria* and *Babesia* parasites. Parasitol Res 91:398–406. https://doi.org/10.1007/s00436-003-0979-2

Schnittger L, Yin H, Jianxun L, Ludwig W, Shayan P, Rahbari S, Voss-Holtmann A, Ahmed JS (2000) Ribosomal small-subunit RNA gene-sequence analysis of *Theileria lestoquardi* and a *Theileria* species highly pathogenic for small ruminants in China. Parasitol Res 86:352–358. https://doi.org/10.1007/s004360050680

Schnittger L, Yin H, Qi B, Gubbels MJ, Beyer D, Niemann S, Jongejan F, Ahmed JS (2004) Simultaneous detection and differentiation of *Theileria* and *Babesia* parasites infecting small ruminants by reverse line blotting. Parasitol Res 92:189–196. https://doi.org/10.1007/s00436-003-0980-9

Schreeg ME, Marr HS, Tarigo JL, Cohn LA, Bird DM, Scholl EH, Levy MG, Wiegmann BM, Birkenheuer AJ (2016) Mitochondrial genome sequences and structures aid in the resolution of Piroplasmida phylogeny. PLoS One 11:e0165702. https://doi.org/10.1371/journal.pone.0165702

Scoles GA, Hutcheson HJ, Schlater JL, Hennager SG, Pelzel AM, Knowles DP (2011) Equine piroplasmosis associated with *Amblyomma cajennense* ticks, Texas, USA. Emerg Infect Dis 17:1903–1905. https://doi.org/10.3201/eid1710.101182

Scoles GA, Ueti MW (2015) Vector ecology of equine piroplasmosis. Annu Rev Entomol 60:561–580. https://doi.org/10.1146/annurev-ento-010814-021110

Sears KP, Kappmeyer LS, Wise LN, Silva M, Ueti MW, White S, Reif KE, Knowles DP (2019) Infection dynamics of *Theileria equi* and *Theileria haneyi*, a newly discovered apicomplexan of the horse. Vet Parasitol 271:68–75. https://doi.org/10.1016/j.vetpar.2019.06.009

Sears K, Knowles D, Dinkel K, Mshelia PW, Onzere C, Silva M, Fry L (2020) Imidocarb dipropionate lacks efficacy against *Theileria haneyi* and fails to consistently clear *Theileria equi* in horses co-infected with *T. haneyi*. Pathogens. 9:1035. https://doi.org/10.3390/pathogens9121035

Sebastian PS, Benitez-Ibalo AP, Flores FS, Debárbora VN, Martinez EI, Thompson CS, Mangold AJ (2021) Molecular detection and phylogenetic characterization of Theileria equi in horses (Equus caballus) from a peri-urban area of Argentina. Ticks Tick Borne Dis 12:101810. https://doi.org/10.1016/j.ttbdis.2021.101810

Sevilla E, González LM, Luque D, Gray J, Montero E (2018) Kinetics of the invasion and egress processes of *Babesia divergens*, observed by time-lapse video microscopy. Sci Rep 8:14116. https://doi.org/10.1038/s41598-018-32349-7

Sevinc F, Sevinc M, Ekici OD, Yildiz R, Isik N, Aydogdu U (2013) *Babesia ovis* infections: detailed clinical and laboratory observations in the pre- and post-treatment periods of 97 field cases. Vet Parasitol 191:35–43. https://doi.org/10.1016/j.vetpar.2012.07.025

Shabangu N, Penzhorn BL, Oosthuizen MC, Vorster I, van Schalkwyk OL, Harrison-White RF, Matjila PT (2021) A shared pathogen: Babesia rossi in domestic dogs, black-backed jackals (Canis mesomelas) and African wild dogs (Lycaon pictus) in South Africa. Vet Parasitol 291:109381

Shaw MK (2003) Cell invasion by *Theileria* sporozoites. Trends Parasitol 19:2–6. https://doi.org/10.1016/s1471-4922(02)00015-6

Sherrill MK, Cohn LA (2015) Cytauxzoonosis: diagnosis and treatment of an emerging disease. J Feline Med Surg 17:940–948. https://doi.org/10.1177/1098612X15610681

Short MA, Clark CK, Harvey JW, Wenzlow N, Hawkins IK, Allred DR, Knowles DP, Corn JL, Grause JF, Hennager SG, Kitchen DL, Traub-Dargatz JL (2012) Outbreak of equine piroplasmosis in Florida. J Am Vet Med Assoc 240:588–595. https://doi.org/10.2460/javma.240.5.588

Sikorski LE, Birkenheuer AJ, Holowaychuk MK, McCleary-Wheeler AL, Davis JM, Littman MP (2010) Babesiosis caused by a large *Babesia* species in 7 immunocompromised dogs. J Vet Intern Med 24:127–131. https://doi.org/10.1111/j.1939-1676.2009.0440.x

Silva VLB, Almeida SLH, Maia MO, Santos TÁ, Pavelegini LAD, Zaffalon GB, Marcili A, Morgado TO, Dutra V, Nakazato L, Pacheco RC (2021) Post mortem protozoan hemoparasites detection in

wild mammals from Mato Grosso state, Midwestern Brazil. Rev Bras Parasitol Vet 30:e013021. https://doi.org/10.1590/S1984-29612021083

Silva JC, Egan A, Arze C, Spouge JL, Harris DG (2015) A new method for estimating species age supports the coexistence of malaria parasites and their mammalian hosts. Mol Biol Evol 32:1354–1364. https://doi.org/10.1093/molbev/msv005

Silva JC, Egan A, Friedman R, Munro JB, Carlton JM, Hughes AL (2011) Genome sequences reveal divergence times of malaria parasite lineages. Parasitology 138:1737–1149. https://doi.org/10.1017/S0031182010001575

Silva MG, Knowles DP, Mazuz ML, Cooke BM, Suarez CE (2018) Stable transformation of *Babesia bigemina* and *Babesia bovis* using a single transfection plasmid. Sci Rep 8:6096. https://doi.org/10.1038/s41598-018-23010-4

Silveira JA, D'Elia ML, de Oliveira AI, de Almeida LR, Dos Santos HA, de Magalhães Soares DF, Ribeiro MF, Dos Santos LW, Ecco R (2016) *Rangelia vitalii* in a free-ranging maned wolf (*Chrysocyon brachyurus*) and co-infections. Int J Parasitol Parasites Wildl 5:280–285. https://doi.org/10.1016/j.ijppaw.2016.09.003

Sivakumar T, Fujita S, Tuvshintulga B, Kothalawala H, Silva SSP, Yokoyama N (2019) Discovery of a new *Theileria* sp. closely related to *Theileria annulata* in cattle from Sri Lanka. Sci Rep 9:16132. https://doi.org/10.1038/s41598-019-52512-y

Sivakumar T, Hayashida K, Sugimoto C, Yokoyama N (2014) Evolution and genetic diversity of *Theileria*. Infect Genet Evol 27:250–263. https://doi.org/10.1016/j.meegid.2014.07.013

Sivakumar T, Igarashi I, Yokoyama N (2016) *Babesia ovata*: taxonomy, phylogeny and epidemiology. Vet Parasitol 229:99–106. https://doi.org/10.1016/j.vetpar.2016.10.006

Sivakumar T, Tuvshintulga B, Kothalawala H, Silva SSP, Lan DTB, Long PT, Ybañez AP, Ybañez RHD, Benitez DF, Tayebwa DS, De Macedo ACC, Schnittger L, Yokoyama N (2020) Host range and geographical distribution of *Babesia* sp. Mymensingh. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13546

Sivakumar T, Tuvshintulga B, Zhyldyz A, Kothalawala H, Yapa PR, Kanagaratnam R, Vimalakumar SC, Abeysekera TS, Weerasingha AS, Yamagishi J, Igarashi I, Silva SSP, Yokoyama N (2018) Genetic analysis of Babesia isolates from cattle with clinical babesiosis in Sri Lanka. J Clin Microbiol 56:e00895–e00818. https://doi.org/10.1128/JCM.00895-18

Šlapeta J, Saverimuttu S, Vogelnest L, Sangster C, Hulst F, Rose K, Thompson P, Whittington R (2017) Deep-sequencing to resolve complex diversity of apicomplexan parasites in platypuses and echidnas: proof of principle for wildlife disease investigation. Infect Genet Evol 55:218–227. https://doi.org/10.1016/j.meegid.2017.09.007

Sloboda M, Jirků M, Lukešová D, Qablan M, Batsukh Z, Fiala I, Hořín P, Modrý D, Lukeš J (2011) A survey for piroplasmids in horses and Bactrian camels in North-Eastern Mongolia. Vet Parasitol 179:246–249. https://doi.org/10.1016/j.vetpar.2011.01.064

Smith MC, Sherman DM (2009) Goat medicine, 2nd edn. Ames, Wiley-Blackwell

Soares JF, Dall'Agnol B, Costa FB, Krawczak FS, Comerlato AT, Rossato BC, Linck CM, Sigahi EK, Teixeira RH, Sonne L, Hagiwara MK, Gregori F, Vieira MI, Martins JR, Reck J, Labruna MB (2014) Natural infection of the wild canid, *Cerdocyon thous*, with the piroplasmid *Rangelia vitalii* in Brazil. Vet Parasitol 202:156–163. https://doi.org/10.1016/j.vetpar.2014.02.058

Soares JF, Girotto A, Brandão PE, Da Silva AS, França RT, Lopes ST, Labruna MB (2011) Detection and molecular characterization of a canine piroplasm from Brazil. Vet Parasitol 180:203–208. https://doi.org/10.1016/j.vetpar.2011.03.024

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

Suarez CE, Alzan HF, Cooke BM (2018) Genomics and genetic manipulation of protozoan parasites affecting farm animals. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 413–438

Sun M, Guan G, Liu Z, Wang J, Wang D, Wang S, Ma C, Cheng S, Yin H, Luo J (2020) Molecular survey and genetic diversity of *Babesia* spp. and *Theileria* spp. in cattle in Gansu Province, China. Acta Parasitol 65:422–429. https://doi.org/10.2478/s11686-020-00179-1

Sun M, Wang J, Liu Z, Guan G, Li Y, Liu J, Xu J, Yin H, Luo J (2019) First molecular evidence of *Babesia occultans* and *Theileria separata* infection in ticks and sheep in China. Exp Appl Acarol 78:223–229. https://doi.org/10.1007/s10493-019-00369-1

Tageldin MH, Al-Kitany Fadiya A, Al-Yahyae Sabra A, Al-I Ismaily SI (2005) Theileriosis in sheep and goats in the Sultanate of Oman. Trop Anim Health Prod 37:491–493. https://doi.org/10.1007/s11250-005-2475-4

Taha KM, Salih DA, Ahmed BM, Enan KA, Ali AM, Elhussein AM (2011) First confirmed report of outbreak of malignant ovine theileriosis among goats in Sudan. Parasitol Res 109:1525–1527. https://doi.org/10.1007/s00436-011-2428-y

Tajeri S, Haidar M, Sakura T, Langsley G (2021) Interaction between transforming *Theileria* parasites and their host bovine leukocytes. Mol Microbiol 115:860–869. https://doi.org/10.1111/mmi.14642

Tamura K, Kumar S (2002) Evolutionary distance estimation under heterogeneous substitution pattern among lineages. Mol Biol Evol 19:1727–1736. https://doi.org/10.1093/oxfordjournals.molbev.a003995

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Theiler A (1901) Die Pferde-Malaria. Schweizer Archiv fuer Tierheilkunde 43:253–280

Tirosh-Levy S, Gottlieb Y, Fry LM, Knowles DP, Steinman A (2020) Twenty years of equine piroplasmosis research: global distribution, molecular diagnosis, and phylogeny. Pathogens. 9:926. https://doi.org/10.3390/pathogens9110926

Tirosh-Levy S, Steinman A, Levy H, Katz Y, Shtilman M, Gottlieb Y (2020) Parasite load and genotype are associated with clinical outcome of piroplasm-infected equines in Israel. Parasit Vectors 13:267. https://doi.org/10.1186/s13071-020-04133-y

Ueti MU, Knowles DP (2018) Equine piroplasmids. In: Florin-Christensen M, Schnittger L (eds) Parasitic protozoa of farm animals and pets. Springer Nature, Berlin, pp 259–270

Ueti MW, Palmer GH, Kappmeyer LS, Statdfield M, Scoles GA, Knowles DP (2005) Ability of the vector tick *Boophilus microplus* to acquire and transmit *Babesia equi* following feeding on chronically infected horses with low-level parasitemia. J Clin Microbiol 43:3755–3759. https://doi.org/10.1128/JCM.43.8.3755-3759.2005

Uilenberg G (1981) *Theileria* species of domestic livestock. In: Irvin AD, Cunningham MP, Young AS (eds) Advances in the control of theileriosis. Nijhoff, The Hague, pp 4–37

Uilenberg G (1999) Immunization against diseases caused by *Theileria parva*: a review. Tropical Med Int Health 4:A12–A20. https://doi.org/10.1046/j.1365-3156.1999.00446.x

Uilenberg G (2006) Babesia—a historical overview. Vet Parasitol 138:3–10. https://doi.org/10.1016/j.vetpar.2006.01.035

Uilenberg G, Andreasen MP (1974) *Haematoxenus separatus* sp. n. (sporozoa, Theileriidae) of sheep in Tanzania. Rev Elev Med Vet Pays Trop 27:459–465

Uilenberg G, Franssen FF, Perié NM, Spanjer AA (1989) Three groups of *Babesia canis* distinguished and a proposal for nomenclature. Vet Q 11:33–40. https://doi.org/10.1080/01652176.1989.9694194

Uilenberg G, Gray J, Kahl O (2018) Research on Piroplasmorida and other tick-borne agents: are we going the right way? Ticks Tick Borne Dis 9:860–863. https://doi.org/10.1016/j.ttbdis.2018.03.005

Uilenberg G, Rombach MC, Perié NM, Zwart D (1980) Blood parasites of sheep in the Netherlands. II. *Babesia motasi* (Sporozoa, Babesiidae). Vet Q 2:3–14. https://doi.org/10.1080/01652176.1980.9693752

Uilenberg G, Schreuder BE (1976) Further studies on Haematoxenus separatus (Sporozoa, Theileriidae) of sheep in Tanzania. Rev Elev Med Vet Pays Trop 29:119–126

Vanstreels RE, Woehler EJ, Ruoppolo V, Vertigan P, Carlile N, Priddel D, Finger A, Dann P, Herrin KV, Thompson P, Ferreira Junior FC, Braga ÉM, Hurtado R, Epiphanio S, Catão-Dias JL (2015) Epidemiology and molecular phylogeny of *Babesia* sp. in Little Penguins Eudyptula minor in Australia. Int J Parasitol Parasites Wildl 4:198–205. https://doi.org/10.1016/j.ijppaw.2015.03.002

Wagner JE (1976) A fatal cytauxzoonosis-like disease in cats. J Am Vet Med Assoc 168:585–588

Walker JB, Keirans JE, Horak IG (2000) The genus *Rhipicephalus* (Acari, Ixodidae). A guide to the brown ticks of the world. Cambridge University Press, Cambridge 643 pp

Wang T, Guan G, Korhonen PK, Koehler AV, Young ND, Hall RS, Yin H, Gasser RB (2017) Mitochondrial genomes of two *Babesia taxa* from sheep in China as a foundation for population genetic and epidemiological investigations. Infect Genet Evol 47:51–55. https://doi.org/10.1016/j.meegid.2016.11.002

Wang JL, Li TT, Liu GH, Zhu XQ, Yao C (2017) Two tales of *Cytauxzoon felis* infections in domestic cats. Clin Microbiol Rev 30:861–885. https://doi.org/10.1128/CMR.00010-17

Wang X, Tedford RH (2008) Dogs: their fossil relatives and evolutionary history. Columbia University Press, New York

Wang X, Wang J, Liu J, Liu A, He X, Xiang Q, Li Y, Yin H, Luo J, Guan G (2020) Insights into the phylogenetic relationships and drug targets of *Babesia* isolates infective to small ruminants from the mitochondrial genomes. Parasit Vectors 13:378. https://doi.org/10.1186/s13071-020-04250-8

Wang X, Wang J, Liu J, Liu A, He X, Xu J, Li Z, Zhao S, Li Y, Yin H, Luo J, Guan G (2019) Comparative analysis of apicoplast genomes of *Babesia* infective to small ruminants in China. Parasit Vectors 12:312. https://doi.org/10.1186/s13071-019-3581-x

Wardrop KJ, Birkenheuer A, Blais MC, Callan MB, Kohn B, Lappin MR, Sykes J (2016) Update on canine and feline blood donor screening for blood-borne pathogens. J Vet Intern Med 30:15–35. https://doi.org/10.1111/jvim.13823

Watts JG, Playford MC, Hickey KL (2016) *Theileria orientalis*: a review. N Z Vet J 64:3–9. https://doi.org/10.1080/00480169.2015.1064792

Werdelin L, Yamaguchi N, Johnson WE, O'Brien SJ (2010) Phylogeny and evolution of cats (Felidae), In: Biology and conservation of wild felids Editors: D.W. Macdonald, A.J. Loveridge Publisher: Oxford University Press, p. 59-82

Wise LN, Kappmeyer LS, Mealey RH, Knowles DP (2013) Review of equine piroplasmosis. J Vet Intern Med 27:1334–1346. https://doi.org/10.1111/jvim.12168

Wong SS, Poon RW, Hui JJ, Yuen KY (2012) Detection of *Babesia hongkongensis* sp. nov. in a free-roaming *Felis catus* cat in Hong Kong. J Clin Microbiol 50:2799–2803. https://doi.org/10.1128/JCM.01300-12

Yabsley MJ, Murphy SM, Cunningham MW (2006) Molecular detection and characterization of *Cytauxzoon felis* and a *Babesia* species in cougars from Florida. J Wildl Dis 42:366–374. https://doi.org/10.7589/0090-3558-42.2.366

Yabsley MJ, Vanstreels RET, Shock BC, Purdee M, Horne EC, Peirce MA, Parsons NJ (2017) Molecular characterization of *Babesia peircei* and *Babesia ugwidiensis* provides insight into the evolution and host specificity of avian piroplasmids. Int J Parasitol Parasites Wildl 6:257–264. https://doi.org/10.1016/j.ijppaw.2017.08.006

Yeruham I, Hadani A, Galker F (1998) Some epizootiological and clinical aspects of ovine babesiosis caused by *Babesia ovis*—a review. Vet Parasitol 74:153–163. https://doi.org/10.1016/s0304-4017(97)00143-x

Yin H, Luo J (2007) Ticks of small ruminants in China. Parasitol Res 101:S187–S189. https://doi.org/10.1007/s00436-007-0688-3

Yin H, Luo J, Guan G, Gao Y, Lu B, Zhang Q, Ma M, Lu W, Lu C, Yuan Z, Guo S, Wang B, Du H, Schnittger L, Ahmed J, Jongejan F (2002) Transmission of an unidentifed *Theileria* species to small ruminants by *Haemaphysalis qinghaiensis* ticks collected in the feld. Parasitol Res 88:S25. https://doi.org/10.1007/s00436-001-0565-4

Yin H, Schnittger L, Luo J, Seitzer U, Ahmed JS (2007) Ovine theileriosis in China: a new look at an old story. Parasitol Res 101:S191–S195. https://doi.org/10.1007/s00436-007-0689-2

Yokoyama N, Okamura M, Igarashi I (2006) Erythrocyte invasion by *Babesia* parasites: current advances in the elucidation of the molecular interactions between the protozoan ligands and host receptors in the invasion stage. Vet Parasitol 138:22–32. https://doi.org/10.1016/j.vetpar.2006.01.037

Young KM, Corrin T, Wilhelm B, Uhland C, Greig J, Mascarenhas M, Waddell LA (2019) Zoonotic *Babesia*: a scoping review of the global evidence. PLoS One 14:e0226781. https://doi.org/10.1371/journal.pone.0226781

Young AS, Mchinja SF (1977) Observations on Haematoxenus separatus Uilenberg & Andreasen 1974 in the erythrocytes of Kenyan sheep. Res Vet Sci 23:387–388

Zahler M, Schein E, Rinder H, Gothe R (1998) Characteristic genotypes discriminate between *Babesia canis* isolates of differing vector specificity and pathogenicity to dogs. Parasitol Res 84:544–548. https://doi.org/10.1007/s004360050445

Zimmermann DE, Penzhorn BL, Vorster I, Troskie M, Oosthuizen MC (2021) *Babesia bicornis, Theileria bicornis* and *Theileria equi* in metapopulations of two black rhinoceros (*Diceros bicornis*) subspecies in South Africa and their potential impact on conservation. Ticks Tick Borne Dis 12:101635. https://doi.org/10.1016/j.ttbdis.2020.101635

Zintl A, Mulcahy G, Skerrett HE, Taylor SM, Gray JS (2003) *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clin Microbiol Rev 16:622–636. https://doi.org/10.1128/CMR.16.4.622-636.2003