Vector-Borne Diseases & Treatment

Chapter 4

Human Babesiosis: Ecoepidemiology, Diagnosis and Treatment

***Lucia Anettová*[1]*; Mária Kazimírová*[1*]**

[1]*Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia.*

[*]***Correspondence to: Mária Kazimírová,** Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia.*

Email: maria.kazimirova@savba.sk

Abstract

Babesiosis is a tick-borne disease of veterinary and medical concern. The disease is caused by parasitic protists of the genus *Babesia* that are transmitted by ticks of the family Ixodidae. In addition to transmission by ticks, several cases of infections through blood transfusion and congenital transmission have been reported in humans. The parasites invade and destroy the erythrocytes of their hosts. In humans, the disease manifestations are broad, from asymptomatic through mild flu-like infection to severe malaria-like disease with a potentially fatal outcome, mainly in immunedeficient and/or elderly individuals. Out of the approximately 100 known *Babesia* species, only a few (*B. microti, B. duncani, B. divergens, B. venatorum*) have been associated with human babesiosis. Babesiosis is endemic in temperate regions of the northern hemisphere. Autochthonous infections in the USA are caused mainly by *B. microti* and *B. duncani.* In Europe, *B. divergens* is the most common etiological agent. In China, several cases of infections with *B. microti* and *B. venatorum* have been reported. Human babesiosis is ranked among emerging diseases. The number of confirmed cases has increased during the last decade, the risk areas have spread and new foci have been discovered**.** As a consequence, babesiosis has become a growing public health challenge, although a considerably high proportion of infections remain asymptomatic or misdiagnosed**.** Changing climate and a number of other biotic and abiotic factors affect the distribution of babesiosis. The infectivity and pathogenicity of the individual *Babesia* species may vary, depending on the strain and geographic region. Accurate

diagnosis of babesiosis is of particular importance as misdiagnosis can lead to confusion with malaria. Filling in the still existing gaps in the knowledge of the life cycle, ecology and distribution of *Babesia* species is essential for the diagnosis and prevention of the disease.

Keywords: *Babesia* Species; Tick-Borne; Emerging Disease; Geographic Distribution

1. Introduction

Babesiosis is a malaria-like zoonotic disease caused by intraerythrocytic parasitic protists of the genus *Babesia* [1,2]. Human babesiosis is endemic in temperate zones of the northern hemisphere, including some regions of North America,Europe and Asia, but can sporadically occur in other parts of the world [2-5]. *Babesia* species causing disease in humans are naturally transmitted via bites of infected ticks, mainly belonging to the genus *Ixodes* (family Ixodidae) [4,5]. Humans can acquire infection also through blood transfusion [6,7] and congenital transmission [8].

"Babesiosis" was named in honour of the Romanian bacteriologist Victor Babeș who first discovered the parasites in 1888 while investigating red blood cells of cattle dying of fever and haemoglobinuria [9]. In 1893, the Americans Theobald Smith and Frederick Kilborne identified the *Boophilus annulatus* tick as the vector of *Babesia bigemina* causing Texas cattle fever. Moreover, this was the first demonstration that an arthropod can act as a disease vector and transmit an infectious agent to a vertebrate host as well as the first observation of transovarial transmission of an infection by a tick female [10]. Following this discovery it had been believed for a rather long period of time that babesiosis could affect only non-human mammals, although *Babesia* parasites were detected in the blood of humans infected with Rocky Mountain spotted fever in Montana in 1904 [11]. The first case of human babesiosis was confirmed in 1957 in a splenectomised herdsman from an area in former Yugoslavia,where cattle were infected with *Babesia bovis* [12]. However, not *B. bovis,* but *B. divergens* was probably the causative agent of the disease [13].

In the 1960s, further cases of human babesiosis were reported in immunocompromised patients from the USA (San Francisco in 1966, the causative agent was not identified [14])and Europe (Northern Ireland in 1967, a fatal case caused by *B. divergens* [15]). In 1969, the first clinical case of babesiosis attributed to *B. microti* was confirmed in an immunocompetent patient with intact spleen from Nantucket Island (Massachusetts, USA) [16] and *Ixodes scapularis* was recognized as the vector [17]. Since then, human babesiosis has been considered an emerging zoonosis with increasing numbers of reported cases in Northern America and a mortality rate of about 5%; majority are caused by *B. microti* in endemic areas in Northeast and the northern Midwest of the USA [1,2,18,19]. Infections with *B. duncani* have been confirmed along the northern Pacific Coast of the USA [20,21] and Canada [22], and a few sporadic infections were caused by *B. divergens*-like parasites [21,23,24]. In Europe, *B. divergens* is the most

common etiological agent. Although the incidence is very low, the disease is very severe [13]. Autochthonous human cases have been registered mainly in immunocompromised patients of which majority have been reported since 1985 [25,26]. In addition to *B. divergens*, infections with *B. microti* and *B. venatorum* (formerly *Babesia* sp. EU1) have been observed [27-29]. Autochthonous cases of human babesiosis caused by *B. microti*-like, *B. ventorum* and *B. divergens* parasites have been reported from China [30-33] and sporadic cases attributed to different *Babesia* species were identified in other parts of the world, e.g. Japan [34-36], Korea [37], Australia [38], South America [39-42] or South Africa [43].

The first report of transmission of *Babesia* by blood transfusion comes from the USA and dates back to 1979 [44]. Since then, over 160 transfusion-transmitted cases have been confirmed, including about 7% fatalities [5]. Majority of the cases were reported in the USA, but also in Europe and Japan[5,7,45,46].

Recent spread of human babesiosis into new areas and emergence of newly described strains and species points to the gaps in the knowledge of the ecology and epidemiology of the disease. Moreover, most cases of babesiosis remain undiagnosed due to the nonspecific clinical signs of the infection. Further investigations of the life cycle, ecology and distribution of *Babesia* species and of clinical presentations of the disease are essential for proper diagnosis and prevention.

2. Transmission Cycle

2.1. Pathogens

Babesia species (family Babesiidae) belong to the phylum Apicomplexa, class Hematozoea, order Piroplasmida, which includes a diverse group of intraerythrocytic parasites called piroplasms (or piroplasmids) due to piriform (peer-shaped) stages occurring in the erythrocytes of their vertebrate hosts [47,48]. Piroplasms (comprising the genera *Theileria, Babesia* and *Cytauxzoon*) are widespread blood parasites of mammals and are transmitted exclusively by hard ticks (Ixodidae) [49,50]. They pose a significant economical, veterinary as well as medical impact. After trypanosomes, babesiae are the second most common blood parasites of free living mammals, but they can infect also birds and lizards [1,51]. Moreover, they cause emerging zoonoses of humans [1,6,13,51,52].

More than 100 *Babesia* species are known to occur worldwide and infect a wide range of free-living and domesticated animals, out of which only a few have caused human babesiosis [1, 18,50]. Along with the progress in microscopic and molecular techniques, the knowledge of *Babesia* is further expanding and probably more species will be discovered [50,53].

2.1.1. Life cycle

Human babesiosis is a zoonotic disease and is acquired through an infectious tick bite when humans accidentally interact with the natural life cycle of the parasite. Humans represent accidental and dead-end hosts for *Babesia* species [3,51].

During the heteroxenous life cycle, *Babesia* parasites undergo developmental changes in the vector and vertebrate host [3,51,54,55]. Generally, asexual reproduction (merogony) takes place in erythrocytes of vertebrate hosts (mainly mammals, to a lesser extent birds and reptiles) infected through an infectious tick bite. The sexual phase of the life cycle (gamogony) and asexual proliferation (sporogony) resulting in the formation of sporozoites, respectively, occur in the midgut and salivary glands of ixodid ticks [48,50,56] [**Figure 1**]. However, the part of the life cycle in the vector has been described for a few species only [48,49] and involves specific modifications, depending on the evolutionary lineages of babesiae [56]. Recent studies using "omics" and systems biology approaches have increased the knowledge of the *Babesia*-tick interactome, but still tick molecules involved in acquisition, transmission and dissemination of the parasite are insufficiently known [57].

During an infectious tick bite, *Babesia* sporozoites are injected together with tick saliva into the vertebrate host where they invade erythrocytes and differentiate into trophozoites. Trophozoites divide asexually (merogony) into two (sometimes four, e.g. in *B. microti* and *B. duncani*; their pattern is referred to as the Maltese cross) merozoites of piriform shape, which invade new red blood cells. Some merozoites stop division and transform into gamonts or pregametocytes [48,50].

After ingestion of infected blood cells by a tick, gamonts differentiate in the tick gut into gametes (ray bodies, Strahlenkorper), which fuse into a diploid zygotes (gamogony). Zygotes undergo meiosis and give rise to primary haploid kinetes. These multiply by sporogony, enter the haemolymph and penetrate different tick organs, including salivary glands and ovaries (in species of the *Babesia* sensu stricto lineage). From tick ovaries, the infection can be transmitted transovarially and infective sporozoites are produced in salivary glands of larvae of the next generation. Kinetes, after invading tick salivary glands, continue their replication until a final cycle of differentiation and multiplication takes place. At this stage, kinetes transform into sporonts which later develop into a sporoblast that is dormant during tick ecdysis (transstadial transmission). During tick feeding that can take several hours or days, maturation of the sporoblast takes place in tick salivary glands and infectious sporozoites are released into tick saliva [48,50,56].

2.1.2. Classification

Several criteria have been adopted in the classification of *Babesia* species. Morphology

and size of *Babesia* has been one of the important and formerly used criteria according to which the parasites were classified as "small" or "large" [1,50]. Molecular methods, however, discovered discrepancies between the morphology of the parasites and DNA sequences and led to their reclassification based on analyses of multiple genes, considering also differences if their life cycle [50,58]. Recent phylogenetic analyses of mitochondrial genome sequences concatenated along with 18S rRNA sequences and analysis of *cox1* amino acid sequences identified five distinct evolutionary lineages in the order Piroplasmida [58]. The analyses confirmed the existence of four previously identified groups (1 - *Babesia* sensu stricto, 2 - *Theileria equi*, 3 - Western *Babesia* group, represented by *B*. *conradae*, 4 - the *Babesia microti* group), but supported the integration of *Theileria* and *Cytauxzoon* species into a single fifth group. These groups are further supported by unique mitochondrial genome structural arrangements and also differ in particular features in their life cycle [56,58].

Babesia species that infect humans originate from three different groups [3,4,21]:

1. *Babesia microti,* which is not a single species, but is represented by a species complex [50,59,60]. Members of this complex are distributed worldwide and infect different hosts (e.g. small rodent, carnivores, macaque, humans). Based on analyses of sequences of 18S RNA, tubulin-beta and CCT7 genes, *B. microti* isolates were found to segregate into different clades (US-type, Kobe-type, Hobetsu-type, Munich-type and isolates from carnivores). The US-type and Kobe-type isolates, respectively, were further divided into three (North America, Western to Central Eurasia, Northeastern Eurasia) and two geographical subtypes (in Japan) and contain zoonotic strains[for review see 50]. Within the rodent isolates from Europe, the zoonotic "Jena" type [29] and the non-zoonotic "Munich" type [3] can be discriminated.

2. Western *Babesia* group that includes *B. duncani* (isolates WA1, WA2, CA5, CA6, CA1, CA3, CA4); they are phylogenetically distinct from *B. microti* and are related to *B. conradae* which infect dogs in the western part of the USA [21,52].

3. *Babesia* sensu stricto. This group includes *B. divergens*, primarily infecting cattle [51], *B. venatorum* infecting roe deer [3,28], and *Babesia* species infecting ungulates, including the KO1 strain[37].

2.2. Vectors and reservoir hosts

Tick species of the Ixodidae family belonging to six genera (*Ixodes, Haemaphysalis, Dermacentor, Hyalomma, Rhipicephalus, Amblyomma*) have been confirmed as natural vectors of *Babesia* parasites[1,61].To date, there is only one report where an argasid tick (*Ornithodoros erraticus)* has been implicated as a vector of *Babesia meri* [62]. Zoonotic *Babesia* species are transmitted mainly by species of the *Ixodes* genus, but for a number of species the vectors and reservoirs are still unknown or suggested [49] [**Table 1**].

The principal vectors of *B. microti* are *Ixodes scapularis* in Northern America [17], *Ixodes ricinus* in Europe [25, 63] and *Ixodes persulcatus* in northeastern Eurasia and Japan [36]. These ticks can harbour and transmit more *Babesia* species and microorganisms that are etiologic agents of zoonotic diseases, such as flaviviruses, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum,* rickettsiae, and other emerging and novel tick-borne pathogens [64]. For example, *I. ricinus* is the known vector of *B. microti*, *B. divergens* and *B. venatorum* [3],but transmits also tick-borne encephalitis virus, *B. burgdorferi* s.l., *Borrelia miyamotoi*, rickettsiae of the spotted fever group, *Coxiella burnetii, A. phagocytophilum, Candidatus* Neoehrlichia mikurensis [65]. Other *Ixodes* species infesting reservoir hosts, including nidicolous species associated with rodents (e.g. *I. angustus, I. eastoni, I. muris,* and *I. spinipalpis* in America and *I. trianguliceps* in Europe) maintain the infections in enzoonotic transmission cycles [49].In addition, congenital infection with *B. microti* was demonstrated experimentally in laboratory BALB/c mice [66] and in wild rodents [67,68], suggesting that vertical transmission of *B. microti* in wild rodent populations may also contribute to the natural cycle. Similar to the vector ticks, natural reservoirs of *B. microti* are also competent reservoirs for other zoonotic pathogens (e.g. *B. burgdorferi*, *B. miyamotoi, A. phagocytophilum*, *Cand.* N. mikurensis), thus coinfections of reservoirs (mainly rodents) frequently occur [49,69].

The ecology, life cycle and interactions of *B. microti* with its vector*, I. scapularis*, have been described in detail and are relatively well understood [70,71]. *Ixodes scapularis* is a three-host tick.Its life cycle typically lasts two years and includes four stages - egg, larva, nymph, and adult. After taking a blood meal, active stages of ticks drop off their hosts and moult into the next stage. Tick larvae and nymphs feed mainly on small mammals or birds, preferably on *Peromyscus leucopus* (white-footed mouse), an important host of the tick and natural reservoir for *B. microti* [71]. In some endemic areas in the USA, up to 60% of white-footed mice and up to 30% of *I. scapularis* nymphs can be infected with *B. microti* [49,61,71,72]. In addition to the white-footed mouse, chipmunks, voles, rats and shrews may serve as competent reservoirs [49,61]. *Ixodes scapularis* females feed on larger hosts, primarily on the white-tailed deer *(Odocoileus virginianus*). This deer species is not a reservoir for *B. microti*, but helps to maintain tick populations and thus contributes to the expansion of babesiosis. Engorged tick females overwinter and lay eggs in the following spring.Larvae hatch in early summer and in late summer they may become infected with *B. microti* through feeding on infected rodents. Engorged larvae overwinter, moult into nymphs in spring of the next year and infest rodents or can accidentally feed on humans during late spring and early summer and infect their hosts. This may be the explanation why most cases of human babesiosis in the USA occur in late spring and summer. In autumn, nymphs moult into adults. These infest white-tailed deer, but can also feed on humans. The following spring, adult female ticks lay eggs that are free of *B. microti* (no transovarial transmission occurs) and the cycle is repeated.

Ixodes pacificus was suggested as the vector of *B. duncani* [22], however, recent findings indicate that *Dermacentor albipictus* may be its vector and the mule deer, *Odocoileus hemionus,* is probably the reservoir host [73]. Lagomorphs are probably reservoirs of *Babesia* sp. MO1 (a species related to *B. divergens*) and *Ixodes dentatus* is likely its vector [49].

In Europe, zoonotic *Babesia* species (*B. divergens, B. venatorum, B. microti*) share the same vector, *I. ricinus* [13,49]. Cattle are natural reservoirs of *B. divergens, B. venatorum* is maintained in cervids (primarily roe deer) and *B. microti* in wild rodents. Transovarial transmission was demonstrated for *B. divergens* and *B. venatorum* [74,75]. Ticks and reservoir hosts infected with the above *Babesia* species have been found throughout Europe, but in general prevalence in ticks is low (0.4 to 2.7%) [49,69]. Prevalence in infected reservoir hosts varies considerably, depending on the location [69,76].Natural infections with *B. microti* have been reported from different rodent and shrew species and prevalence in rodents can reach locally up to 40% [49,69]. However, there are indications that zoonotic and non-zoonotic *B. microti* strains co-circulate in the same rodent species [77].

In Asia, rodents and shrews are likely reservoirs of the zoonotic *B. microti* Kobe-type, *B. microti* Hobetsu-type and *B. microti* US-type parasites [49]. The vector of *B. microti* Hobetsu-type in Japan is probably *I. ovatus*. *Ixodes persulcatus* is the suggested vector of *B. microti* US- type and *B. microti* Kobe-type in Russia and China (reviewed in [30,35,36,49]).

3. Geographic Distribution and Epidemiology

During the past decades, the number of human babesiosis cases has increased in many parts of the world. Higher incidence rates in endemic areas, expansion in geographic distribution, rise in new areas, increasing numbers of imported cases due to travel, and new *Babesia* strains infecting humans have been reported [49]. Due to a relatively large proportion of asymptomatic infections (supported by results of serological surveys) and misdiagnoses, the number of actual cases is probably underestimatedand may be even higher. In addition, asymptomatic carriers of the parasites being blood donors represent a great risk for blood transfusion. Thus, babesiosis is recognized as an emerging health risk worldwide [2, 49].

3.1. North America

United States

Most of babesiosis cases in the USA are attributed to*B. microti* which was first identified in 1966 [16]. Babesiosis is found primarily in the northeastern and midwestern states, but its geographic range is expanding [71]. However, the knowledge of the current geographic distribution of babesiosis is incomplete as it relies only on reports of human cases [72]. In 2011, human babesiosis became a nationally notifiable disease to the U.S. Centers for Disease Control

and Prevention [http://wwwn.cdc.gov/nndss/conditions/babesiosis/case-definition/2011/]. Over the past decade, increasing incidence of babesiosis has been demonstrated [71]. Particularly, since 2011 to 2017 the number of reported cases increased from 1,126 to 2,368, and the disease was reported from 30 states in 2017 [78-80]. However, vector-based surveillance of babesiois in endemic sites suggests that the disease is underreported [72]. Clinical manifestations of the disease range from asymptomatic to multiorgan failure. Severe illness occurs mainly in elderly, immunocompromised, and asplenic patients [4]. Fatality rates of about 20% have been reported among immunosuppressed patients or in those who were infected by transfusion [72]. Enhanced clinical and public awareness,changes in tick, deer, and rodent populations related to climate change and deforestation are probably factors affecting the increases in diseaseincidence and its geographic expansion [64,81,82].

Ixodes scapularis, the primary vector of *B. microti* is also an important vector for other tick borne disease agents, *B. burgdorferi* and *A. phagocytophilum*, the causative agents of Lyme disease and human granulocytic anaplasmosis, respectively. The geographic distribution of babesiosis, however, is more limited than that of Lyme disease [71,72]. In addition, other human disease agents such as Powassan virus, *Ehrlichia muris eauclarensis, Borrelia miyamotoi*, and *Bo. mayonii*, are transmitted by *I. scapularis* and some of them share reservoir hosts [64, 72,82]. Co-infections of field-collected ticks with these agents are common and humans can contract more infections simultaneously through a single tick bite or from concurrent bites by singly-infected ticks [83]. These conditions can increase the severity of the diseases andmake its diagnosis difficult [82, 84-86].

Sporadic human cases of babesiosis, partly severe to fatal in immunocompromised individuals, have been attributed to*B. divergens*-like species [24] and *B. duncani* [21]. *B. divergens*-like organisms (currently designated as *Babesia* species MO1) have caused severe disease, e.g., in Kentucky, Missouri, Washington State [23,24] and recently in Michigan [87]. *Babesia duncani* (formerly WA1) [21] infections have been reported along the Pacific coast, in Washington State [88] and disease due to *Babesia* sp. CA-type (known as CA1–CA4, related to *B. duncani*) in California [20].

Transfusion-associated babesiosis represents a serious issue in the United States. Over 160 cases have been identified, which are often severe, sometimes fatal [46,71]. Majority of cases were attributed to *B. microti* and only three cases to *B. duncani.*

Canada

Babesiosis is considered an emerging disease in Canada. Autochthonous infections with *B. duncani* and *B. microti* have been identified in Canadian patients [89,90]. During a recent surveillance study of human *B. duncani* infectionsconducted from 2011 to 2017,1,119 cases were identified. The study revealed that *B. duncani* infections were distributed in 10 provinces,

with the highest occurrence at the Pacific coast. The symptoms ranged from mild to fatal [22]. The vector of *B. microti* is probably *I. scapularis*, but the vector of *B. duncani* has not been confirmed.

3.2. Europe

Over 50 autochthonous cases of human babesiosis have been reported in different European countries since the first evidence of the disease in 1957 [examples are presented in Table 1]. Most clinical cases have been associated with the bovine parasite *B. divergens,* but since 2003 a few cases have been attributed to *B. venatorum* (formerly EU1) and *B. microti* [3,28,29, 51,91,92]. However, serosurveys indicate that part of the infections remains asymptomatic and/or undiagnosed. Depending on the selected cohort and area, seroprevalence of *Babesia* antibodies was found between 2% and 23% [93-95]. Recent seroscreening of patients with history of tick bite in Belgium detected positive reactions in 9% patients against *B. microti*, 33% against *B. divergens* and 40% against *B. venatorum*[96]. In another study including *B. burgdorferi*-infected individuals in Sweden, 16.3% and 2.5% of *Borrelia*-infected and healthy persons, respectively, were seropositive to *Babesia* spp. [97].

The clinical signs of babesiosis caused by *B. divergens* and *B. divergens*-like organisms are usually severe, in some cases fatal, and are limited mainly to splenectomised and immunocompromised patients including HIV-positive individuals [13,98]. However, disease has been diagnosed also in a few immunocompetent patients; one from France [99], two from Spain [26,100]. About 50 clinical cases attributed to *B. divergens* have been reported till 2017 [25, 26]. Out of the 9 cases reported from 2003 to 2017, three cases (in Finland, Portugal, and Spain) were fatal [26]. The geographic distribution of the parasite co-incides with the distribution of infected cattle populations and the occurrence *I. ricinus*-infested areas [13].

Babesia venatorum was implicated in the first reported cases of human babesiosis in Italy, Austria, and Germany [28,92] and in a recent case in Sweden[101]. This species is closely related to the *B. odocoilei/B. divergens* complex [28]. *B. venatorum*-infected *I. ricinus* ticks and wild-living ruminants have been found in many European countries, showing a wide geographic distribution across the continent. These findings, together with increasing tick and roe deer populations and their occurrence in urban parks suggest that the parasite is another candidate for the emergence of a new zoonotic tick-borne disease in Europe [65].

As mentioned above, *B. microti*infections of humans show a wide range of symptomsand occur mainly in the United States. Only one autochthonous case of human babesiosis attributed to this parasite in a patient from Germany who was probably infected through transfusion [29] and two asymptomatic infections in Poland [102] are known from Europe. Majority of the other diagnosed *B. microti* infections were imported and involved travellers from the USA [25, 103,104] [see examples in **Table 1**]. The parasite has been detected in *I. ricinus* ticks and

wild rodents throughout Europe, with varying prevalence rates [e.g. 65,69]. These findings, together with results of serosurveys (seroprevalence rates between 1.5-9%) [96,97,103,105], suggest that there is a growing risk of locally acquired *B. microti* infections, mainly in humans exposed to tick infestations.

3.3. Asia and other parts of the world

Babesia microti–like parasites were identified as causative agents of babesiosis in Japan [34]. The first symptomatic infection in Japan, documented in 1999, was transfusion-acquired and was caused by the *B. microti*-like Kobe-type parasite [106]. Subsequently, parasites belonging to another parasite (designated as the Hobetsu-type) were isolated from wild rodents in various places in Japan [35] and in *I. ovatus* ticks [107], while*B. microti* US lineage was detected in *I. persulcatus* [36,107]. These results, together with a retrospective seroepidemiological survey for human babesiosis suggest that babesiosis in Japan occurred prior to the discovery of the first clinical case [108] and infections in humans might have been undetected.

A case of human babesiosis was detected in South Korea and was caused by the newly identified KO1 strain that is related to a species found in sheep [37].

Human babesiosis attributed to *B. microti*–like parasites was reported from Taiwan province [109], from different regions and provinces in mainland China [30,33] and from Hong-Kong [110] [**Table 1**]. In China, babesiosis is not endemic, but is considered an emerging parasitic disease, with 75 human casesdiagnosed till 2016 [32,33,111]. Results of a preliminary screening of limited sample of blood donors showed a 1.3% seropositivity for *B. microti* [112] and suggest that increased attention should also be paid to the risk of transmission through transfusion. In addition to *B. microti*, the other species causing infections are *B. venatorum* and *B. divergens* [30,113,114]. The suggested vectors of these parasites are *I. persulcatus* and *Haemaphysalis concinna*. The recently discovered *Babesia* sp. XXB/HangZhou is a novel *Babesia* species clustering with *B. microti*, which caused mild babesiosis in an immunocompetent patient [115]. *Babesia crassa*-like infections were detected in *I. persulcatus* and *H. concinna* ticks, sheep and for the first time in humans in northeastern China [116]. The infections were mild to moderate, but there is still a risk of severe illness in immunocompromised people.

Human babesiosis has not been reported so far from Mongolia, but *B. microti* infections were confirmed in blood of farmers in Selenge province near the Russian border - 7% by indirect fluorescent antibody (IFA) test and 3% by PCR [117].

Sporadic cases of babesiosis have been reported in other parts of Asia (India [118], Quatar [119]), in Africa (Egypt, pet associated [120], Equatorial Guinea, caused by *B. microti*,

not clear if autochthonous [121], South Africa [43]) and in Australia, attributed to *B. microti* [38] [**Table 1**]. Severe illness attributed to *B. divergens* was diagnosed in a splenectomised patient in Turkey [122].

A few cases of uncharacterized babesiosis have been reported in Brazil [39] and Colombia [40], and cases attributed to *B. microti* have been identified in Ecuador [123] and Mexico [42]. However, human babesiosis is likely underestimated in South America. For example, among asymptomatic individuals from two rural communities in southeastern Bolivia, blood of 3.3% persons tested positive for *B. microti* (US lineage) DNAby PCR and seroprevalence was 45.7% [41].

4. Infections in Humans, Pathogenesis and Clinical Manifestations

The most common ways of transmission of babesiosis are tick bites and contaminated blood. Transplacental transmission is also possible [5]. Incubation period ranges from 1 to 6 weeks, most often 2 to 4 weeks. In this period, general discomfort and weakness usually occur.

4.1. Pathogenesis

The clinical and laboratory signs of human infection with *Babesia*parasites are mostly related to development of piroplasms in erythrocytes, where differentiated and undifferentiated trophozoites are produced. The exact haemolysis mechanism is unknown, but it can be related to trophozoites leaving the red blood cell and damaging the membrane. Invaded erythrocytes increase their adherence to capillary walls and decrease their deformability, which can cause respiratory complications like noncardiac pulmonary oedema and acute respiratory distress syndrome [124-126].After erythrocyte destruction, the result of which is haemolytic anaemia, fragments may cause capillary blockage in the spleen, liver, kidneys and central nervous system. Many of the most common clinical features, such as fever or pain of the muscles and joints, are caused by macrophage-produced mediators, mainly interleukin-1 and tumour necrosis factor, provided that the complement system is activated. Incidence and severity of babesiosis are usually higher, as mentioned before, in splenectomised patients, because the spleen and cell-mediated immune responses provide an important defence mechanism against piroplasmosis. The main role of the spleen is the removal of infected erythrocytes from circulation, which are subsequently ingested by macrophages [126].

4.2. Laboratory changes

Babesia infections present themselves with several typical laboratory changes. In the haematological profile, decreased haematocrit due to haemolytic anaemia is observed. Other frequent indicators include thrombocytopenia and leukopenia [52,99]. Blood biochemistry

profile often shows elevated hepatic transferases like alanine-aminotransferase and aspartate-aminotransferase. Blood urea nitrogen (BUN) and serum creatinine also reach higher levels.

4.3. Clinical manifestation

Human babesiosis can have a wide range of symptoms and its severity may range from an asymptomatic infection to a life-threatening disease.

Mild to moderate infection

In mild to moderate infection, only flu-like symptoms like loss of appetite, nausea or headache may be present, therefore many infected individuals do not seek medical consultation and infections stay undiagnosed. Most common clinical signs are fatigue, headache, myalgia and arthralgia, intermittent fever and anorexia. Abdominal pain, weight loss, vomiting and emotional lability may also be present [127]. Beside fever, clinical findings also include splenomegaly and hepatomegaly, retinal haemorrhages, petechiae, ecchymoses, or less commonly rash. Due to haemolysis, jaundice and dark urine may occur. Parasitaemia may persist after the disappearance of clinical manifestations and in these cases it usually lasts several weeks to several months. Mild to moderate infection is usually caused by *B. microti* in the USA [52], mostly in immunocompetent individuals with no concurrent infection.

Severe infection

Even though *B. microti* infections usually result in mild illness, they may be severe or fatal in splenectomised or immunosuppressed patients, and increased odds of serious clinical and pathological changes were observed in elderly persons as well [128]. This underlines the need of considering the vulnerability of the patient in prognosis and treatment. In infections with other *Babesia* species, especially *B. divergens, B. duncani* and *B. venatorum*, there seems to be a higher probability of severe disease [18,84].

In severe cases of babesiosis, complications often appear. As mentioned earlier, the most common complication is acute respiratory failure [124-126,129].Beside respiratory complication, renal or liver failure are quite common. Disseminated intravascular coagulation and congestive heart failure may also occur [130]. Due to anaemia, low or unstable blood pressure is sometimes detectable. Autoimmune disorders are sometimes associated with babesiosis, of which autoimmune haemolytic anaemia is the most often described one [131]. Also immune thrombocytopenia is reported occasionally, but even though thrombocytopenia is a common finding in human babesiosis, the exact mechanism still needs to be characterized [132].

Asymptomatic parasitaemia

Asymptomatic parasitaemia may persist for months to years after the symptoms of illness have disappeared. Many people infected with *B. microti* have never developed any symptoms [127, https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf]. Asymptomatic babesial infections are identified in serosurveys mostly in United States. In endemic regions, a high percentage of *B. microti* infections may be asymptomatic [19], and therefore a considerable risk of transmission of the infection exists via blood transfusion from blood donors with asymptomatic parasitaemia.

5. Diagnosis

First of all, there should be a suspection of the disease in febrile patients or if other symptoms described earlier occur, especially those more specific for babesiosis, like haemoglobinuria or splenomegaly and if there is an anamnesis of tick bite 1 to 6 weeks ago or blood transfusion in the previous 6 months. Nevertheless, there are cases of individuals diagnosed with babesiosis who did not recollect being bitten by a tick [22], so this should be considered regardless of this circumstance.

There are several methods of diagnosing babesiosis in humans.

Microsopic methods

In the phase of acute parasitaemia, the easiest and also most widely used method once a blood parasite is suspected is microscopic observation of piroplasms within erythrocytes in a blood smear. Giemsa or Wright stains are usually used [52]. This method does not provide other than morphological criteria and most species are morphologically indistinguishable. It is possible to categorize parasites as „large“ or „small“ *Babesia* [49,54]. Small babesiae include *B. bovis, B. divergens* or *B. microti* and others and their merozoites are smaller than erythrocyte radius [54]. On the other hand, *B. bigemina, B. canis* and *B. major*, none of which causes a human infection,belong to large babesiae whose merozoites are longer than erythrocyte radius. Merozoites of *Babesia* spp. could resemble *Plasmodium falciparum* trophozoites, so a combination of criteria has been developed to help distinguish *Babesia* spp. from *Plasmodium*. It includes absence of hemozoin, typical „Maltese–cross” appearance and presence of extracellular forms, although these manifestations cannot be seen very often [129]. The Maltese–cross pattern can be observed especially in *B. microti* or *B. duncani*, rarely in other species [133]. *B. divergens* and *B. venatorum* merozoites typically appear as paired pear-shaped forms [127].

Another microscopic technique, although much less widely used, is a haemolymph test. In this test haemolymph smear is obtained by cutting off a tick’s leg with small scissors and

dropping a small amount of haemolymph on the slide [134]. As well as in blood smear, Giemsa stain can be used. In the haemolymph smear, kinetes, motile stages of the parasites, which appear 72 hours after feeding, can be observed.

Microscopy of thin blood smear is sensitive enough only in the acute stage, when parasitaemia is high. To confirm chronic infection, methods of serology or molecular biology are required.

Serology

In chronic cases of human babesiosis, or when parasitaemia is low, serological tests are very useful. These immunological techniques detect specific antibodies against protein blood stages. One of the most widely used methods is indirect immunofluorescence assay (IFA) [135]. This method provides very good specificity and sensitivity and it has been developed for all *Babesia* species [134]. However, when an early diagnosis is made in the initial stage of the infection, the serum may be sampled before the production of antibodies, therefore false negative results may occur [136]. Another issue is that there is currently no standardised IFA procedure for diagnostic laboratories, so the interpretation often strongly depends on the investigator's experience and other factors, e.g. type of conjugate used or preparation of the antigen [25,137]. In *B. microti*, it was determined that a titre of ≥1:1024 indicates active or recent infection and usually returns to ≤1:64 within 8 to 12 months [18]. In the case of *B. divergens* there is insufficient data for determining titres due to the small number of available clinical human samples with confirmed *B. divergens* infection [25].It is possible to use IgM antibodies to differentiate recent from past infection since IgM antibodies are usually present in the first stage after the clinical signs appear [127,138].

The currently available serological techniques for diagnosing babesiosis in humans also include enzyme linked immunosorbent assay (ELISA), immunoblot or immunochromatography (ICT), but these are not standardised for routine use in diagnostic laboratories yet [103,134, 139]. Especially ELISA seems to be a distinctly time-saving immunological method, because large amounts of samples can be screened at once and they are not influenced by the personnel's subjectivity. In 2018, Thekkiniath et al. [140] described a novel antigen capture ELISA method with very high sensitivity, detecting BmGPI12 protein of *B. microti*. According to another 2018 study by Cai et al. [33], ELISA test using rBmSA5-1-1 protein demonstrates high specificity and sensitivity for the serologic detection of *B. microti* in mice and it also confirms suitability of ICT in rapid diagnostics of *B. microti* infection. ICT should be considered as an immunological method of the future, since it provides good specificity and it has much lower costs than other techniques that require trained staff or special equipment (e.g. IFA). However, no ICT kit seems to be commercially available as of December 2018 [134,139]. Highly specific monoclonal antibodies designed to identify parasites directly from the blood still need to be

developed [134].

Molecular methods

In both acute and chronic infections it is possible to diagnose babesiosis using the methods of molecular biology such as polymerase chain reaction (PCR) or real-time (RT) PCR, which provide high levels of specificity and sensitivity. Just like microscopy, these are direct methods of parasite detection, but they are much more sensitive and they make the parasites detectable also in stages with very low parasitaemia or presence of antibodies, which is a scenario where both microscopy and serology often fail.

Human EDTA-stabilized whole blood may be used in this technique. The most commonly employed target in the detection of *Babesia* spp. is 18S rRNA [5,133,136].In *B. microti*, the lower limit for detecting the pathogen is about 100 gene copies, which means approximately 5-10 parasites in μL [5].Subsequently, it is preferred to sequence the PCR product.

Nowadays new molecular assays are in development, mostly for *B. microti*. RT-PCR using new targets instead of today used 18S rRNA gene seems to be a promising way to enhance the detection of parasites. In the study performed by Grabias et al. [141], an abundant BMN antigen family used to diagnose *B. microti* infection in mice blood was more effective and sensitive compared to 18S rRNA.

6. Therapy and Prevention

Tick management

Avoidance of tick bites seems to be the most important step in the prevention of all tick-borne diseases, not only piroplasmosis. In the case of blood protozoa, pathogens can be transmitted from tick saliva to human blood approximately after 48 hours of tick feeding, so early tick removal may also help minimalize the risk of infection. This, however, differs in tick-borne bacteria and viruses.

Antiprotozoal treatment

The apicoplast, a secondary endosymbiotic organelle of the phylum Apicomplexa, has recently been an attractive target for the development of antiparasitic drugs for apicomplexan parasites [134]. As a matter of fact, several currently used antimicrobials (e.g. clindamycin and macrolide antibiotics) do inhibit the replication of this plastid in eukaryotic microorganisms [142] and since it is important for the survival of the parasite, aiming the antiparasitic therapy at the apicoplast seems to be an effective way of pathogen elimination. Although several other studies about anti-plastid drugs in animals and humans were carried out [143,144], further research of the apicoplast as a target of antiparasitic therapy is needed, particularly with regard

to babesiae.

Nowadays, other approaches are in research, since presently available management strategies still do not provide complete recovery in immunosuppressed individuals [145] and also bring certain risks of toxicity or raising parasite resistance [56,146]. Proteasome inhibitors used as anti-cancer drugs are very promising new candidates in the treatment of babesiosis [56]. According to a 2018 study by Jalovecka et al. [56], carfilzomib seems to be effective on *ex vivo* bovine blood culture and also *in vivo* on mice without apparent adverse effects.

Several approaches in the management of human babesiosis have been described. In mild to moderate cases mostly caused by *B. microti*, the combination of atovaquone (750 mg orally twice a day) and azithromycin (first day 500-1000 mg orally and subsequent days 250-1000 mg) administered over the period of 7 to 10 days seems to be as effective in reducing symptoms and clearing parasitaemia as its widely used predecessor, the combination of clindamycin and quinine, and causes fewer adverse effects [133,147, https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf]. In immunocompromised patients, 6 weeks of therapy with higher doses of azithromycine may be needed [148]. However, further research is still needed for the therapy in immunosuppressed patients, since there are cases of individuals for whom this regimen did not prove to be completely curative [145,146].

In the alternative approach, quinine (650 mg orally 3 times a day) and clindamycin (600 mg orally 3 times a day or 300–600 mg intravenously 4 times a day) are used for 7-10 days [139, https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf].Although this regimen is associated with more adverse effects like gastroenteritis or tinnitus [18], according to many authors, quinine plus clindamycin should be used in severe infections with babesiae rather than other protocols, since their effectiveness was proven in *B. duncani*, *B. divergens* and *B. divergens*-like infections and when severe clinical changes were present in patients [18, 103,149].In infants, the protocol of atovaquone and azithromycine appears to be both effective and safe [150, https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf]. Other antimalarial drugs such as chloroquin are not recommended in cases of human babesiosis, although there have been studies confirming the effectiveness of antitrypanosomal drugs in the treatment of piroplasms, namely the combination of pentamidine and cotrimoxazole [129,139,151,152]. However, due to renal toxicity, pentatimidine is not considered suitable for the treatment of human babesiosis, especially in individuals with severe haemolytic anemia [13].

Supportive treatment

In the cases of clinical babesiosis, especially in severe infections with grave clinical symptoms and status, additional therapy is needed. Fluid therapy and/or blood transfusions may be necessary. The use of anti-inflammatory drugs is appropriate, but doses need to be considered carefully due to renal toxicity, especially in dehydrated or haemolytic patients.

In addition, nutritional supplements such as iron, dextrose and vitamins (B complex) may be beneficial.

To date, there is no commercially available possibility of active immunisation against ticks or babesiosis in humans, but there are results in this field that deserve attention. Nowadays, a number of tick salivary proteins are considered to be promising new anti-tick vaccine candidates due to their antigenic features [153,154]. For relevant research in this field, although not specifically related to babesiae, but rather to other tick borne pathogens; see [155-157], and several others mentioned in the review by Neelakanta and Sultana [158]. In general, transmission-blocking vaccines such as anti-vector vaccines are targeting vector molecules in order to block pathogen transmission from tick (vector) to mammalian host [158]. For babesiae, extensive research and description of extracellular proteins of *B. microti* which can be used as vaccine candidates or diagnostic serological markers was done by Elton et al. [159]. Apparently, these proteins are an effective target, because they are directly accessible to host immune system and importantly, high titres of antibodies against *B. microti* major coat protein did not turn out to affect parasitaemia or pathology of the infection in mice [159].

7. Conclusions

Tick-borne piroplasms pose a significant burden on human and animal health worldwide. Human babesiosis is considered an emerging disease, because the number of cases and the geographic area of their distribution have dramatically increased over the past decade. Moreover, new species and strains of known zoonotic *Babesia* species are continuously discovered and appear in new geographic areas. However, the ecology, vectors and reservoirs are known only for a few zoonotic *Babesia* strains. Infectious tick bites and blood transfusion represent the main routes of infections. Babesiosis has probably been overlooked or misdiagnosed in many parts of the world due to the lack of public and medical awareness and insufficient detection methods. More advanced detection methods and molecular typing are needed to characterize the pathogens associated with human disease. Further epidemiologic studies, serosurveys and molecular studies are necessary to reveal the current distribution of endemic areas of zoonotic species and strains. Research is also needed to better characterize the pathogenesis of the disease, particularly in cases when co-infections by other tick-borne pathogens and/or immunocompromised or patients are concerned. Diagnostic tests should be improved by involving molecular and more advanced immunological methods, particularly in cases when conventional light microscopy fails. Diagnostic tests should be developed and applied to screen donors and blood supply in order to prevent transfusion-transmitted babesiosis. Optimising of treatment regimes, their duration and therapeutic combinations is inevitable, particularly in severe cases of babesiosis in immunocompromised patients. Increased public awareness of the disease and effective prevention measures including anti-tick and transmission blocking vaccines should be involved to control the disease.

Table 1: Geographic distribution, vectors and reservoir hosts of *Babesia* species infecting humans
Notes: Not all imported cases in Europe and in other parts of the world are listed. *ni* - vector and / or host was not identified. For *B. divergens* infections, fields for vectors and reservoirs are empty; in some cases they were not identified, in other cases *I. ricinus* and cattle are suggested as the vector and reservoir, respectively.

Distribution	*Babesia* species	Vectors	Main reservoirs	Reference
North America				
USA (northeast, upper Midwest, and mid-Atlantic)	*B. microti*	*Ixodes scapularis*	rodents, shrews	[2, 49, 71, 78]
Missouri, Kentucky, Washington, Massachusetts Michigan	*Babesia* sp. MO1, *divergens*–like	*Ixodes dentatus*	Lagomorphs	[23, 24, 87]
Washington state	*B. duncani* (formerly *Babesia* sp. WA1)	*Dermacentor albipictus*	*Odocoileus hemionus*	[73, 88]
California	*Babesia* sp. CA- type(CA1-4), related to *B. duncani*	*ni*	*ni*	[20]
Canada	*B. microti*	Probably *I. scapularis*	*ni*	[90] .
	B. duncani			[22]
Europe				
Germany	*Babesia microti*	*Ixodes ricinus*	rodents	[29]
Poland	*B. microti*, asymptomatic	*ni*	*ni*	[102]
Switzerland	*Babesia microti*	*I. ricinus*	rodents	[105]
Spain	*Babesia microti*, imported			[160]
France	*B. microti*, imported			[161]
Poland	*B. microti*, imported			[162, 163]
Czech Republic	*B. microti*, imported			[164]
Denmark	*B. microti*, imported			[165]
Belgium	*Babesia* sp., serosurvey			[96]
Former Yugoslavia	*B. divergens*	*I. ricinus*	cattle	[12]
Spain	*B. divergens*			[26, 98, 100, 166]
Northern Ireland	*B. divergens*			[15, 167]
Finland	*B. divergens*			[168]
Norway	*B. divergens*			[169]
France	*B. divergens*			[99, 170]
Canary Islands, Spain	*B. divergens*-like	*Ixodes ventalloi*?	*ni*	[171]
Portugal	*B. divergens*-like	*ni*	*ni*	[172]
Sweden	*B. divergens*			[173]
Sweden	*B. venatorum* ; antibodies to *B. microti* and *B. divergens*			[97, 101]
Austria, Italy,	*B. venatorum*	*I. ricinus*	roe deer	[28]
Germany	*B. venatorum*			[92]

Eurasia, Asia				
Russia	*B. bovis? B. divergens*	probably *I. persulcatus*	rodents	[174, 175]
Turkey	*B. divergens?*	*ni*	*ni*	[122]
Japan	*B. microti*-like, probably Kobe type	*ni*	*ni*	[106,176]
	B. microti US lineage	*I. persulcatus*	rodents	[36, 107]
	B. microti-like Kobe-type	*ni*	rodents	[34]
	B. microti-like Hobetsu-type	*I. ovatus?*	rodents	[35, 107, 177]
Mongolia	*B. microti* serosurvey	*ni*	*ni*	[117]
Taiwan	*B. microti*-like	?	?	[109]
China	*B. microti*	probably *I. persulcatus, Haemaphysalis concinna*	rodents	[30, 111]
	B. venatorum	probably *I. persulcatus*, *H. concinna*	*ni*	[114, 178]
	B. divergens	probably *I. persulcatus, H. concinna*	*ni*	[113]
	Babesia sp. XXB/HangZhou	*ni*	*ni*	[115]
China-Myanmar border	*B. microti*			[31, 179]
	Babesia crassa-like	probably *I. persulcatus, H. concinna*	probably sheep	[116]
Hong Kong Pet-associated	*Babesia* sp.?, probably *B. microti*	*ni*	*ni*	[110]
Korea	*Babesia* sp. KO1	*ni*	probably sheep	[37]
India	*Babesia* sp.	*ni*	*ni*	[118]
Qatar	Babesia sp.; introduced			[119]
South America				
Brazil	*B. microti*	*ni*	*ni*	[39]
Columbia	*B. microti*	*ni*	*ni*	[40]
Bolivia	*B. microti*	*ni*	*ni*	[41]
Mexico	*B. microti*	*ni*	*ni*	[42]
Ecuador	*B. microti*	*ni*	*ni*	[123]
Africa				
Egypt	*Babesia* sp.	*ni*	*ni*	[120, 180]
Equatorial Guinea	*B. microti*; not clear if autochthonous	*ni*	*ni*	[121]
South Africa	*Babesia* sp.	*ni*	*ni*	[43]
Australia	*B. microti*	*ni*	*ni*	[38]

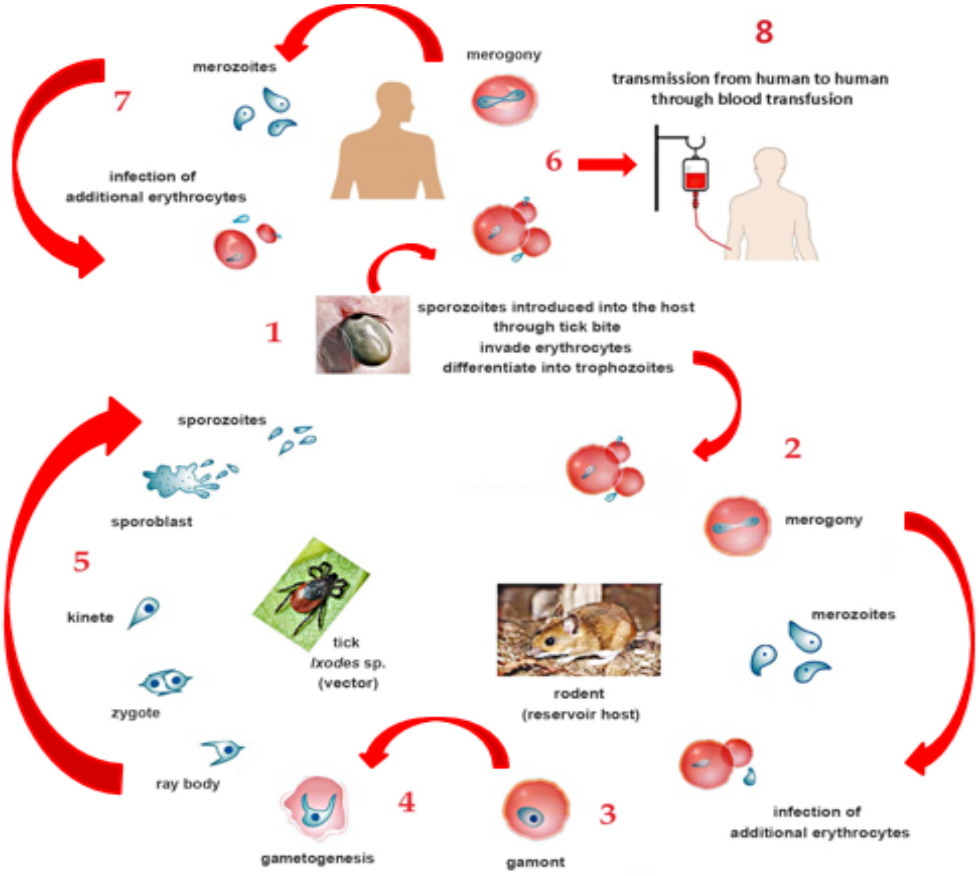

Figure 1: *Babesia* sp. life cycle. **1.** Infected ticks feed on mammalian hosts and inject *Babesia* sporozoites into the bloodstream together with saliva. **2.** Sporozoites invade erythrocytes and differentiate into trophozoites which divide asexually into two (sometimes four merozoites) (merogony). Merozoites destroy erythrocytes and infect additional erythrocytes**. 3.** In a reservoir host (rodent), a few merozoites transform into gamonts. **4.**When an ixodid tick feeds on an infected competent reservoir host, gamonts are taken up and differentiate in the gut of the ticks into gametes (ray bodies), they fuse and become diploid zygotes. **5.** Zygotes undergo meiosis and give rise to haploid kinetes. Kinetes multiply by sporogony and invade the hemolymph and subsequently several tick organs, including salivary glands. In the salivary glands, kinetes transform into infectious sporozoites that are transmitted to a mammalian host through a tick bite. Humans are generally intermediate or dead end hosts of *Babesia* spp. In humans, **6.** merogony, and **7.** infection of additional erythrocytes take place. **8.** Human-to human transmission can occur through bloodtransfusion. Modified based on https://commons.wikimedia.org/wiki/File:Babesia_life_cycle_human_en.svg#/media/File:Babesiosis_life_cycle_without_text.png

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