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Review

Natural history of Zoonotic *Babesia*: Role of wildlife reservoirs

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ABSTRACT

Babesiosis is an emerging zoonotic disease on all inhabited continents and various wildlife species are the principal reservoir hosts for zoonotic *Babesia* species. The primary vectors of *Babesia* are Ixodid ticks, with the majority of zoonotic species being transmitted by species in the genus *Ixodes*. Species of *Babesia* vary in their infectivity, virulence and pathogenicity for people. Various factors (e.g., increased interactions between people and the environment, increased immunosuppression, changes in landscape and climate, and shifts in host and vector species abundance and community structures) have led to an increase in tick-borne diseases in people, including babesiosis. Furthermore, because babesiosis is now a reportable disease in several states in the United States, and it is the most common blood transfusion-associated parasite, recognized infections are expected to increase. Because of the zoonotic nature of these parasites, it is essential that we understand the natural history (especially reservoirs and vectors) so that appropriate control and prevention measures can be implemented. Considerable work has been conducted on the ecology of *Babesia microti* and *Babesia divergens*, the two most common causes of babesiosis in the United States and Europe, respectively. However, unfortunately, for many of the zoonotic *Babesia* species, the reservoir(s) and/or tick vector(s) are unknown. We review the current knowledge regarding the ecology of *Babesia* among their reservoir and tick hosts with an emphasis of the role on wildlife as reservoirs. We hope to encourage the molecular characterization of *Babesia* from potential reservoirs and vectors as well from people. These data are necessary so that informed decisions can be made regarding potential vectors and the potential role of wildlife in the ecology of a novel *Babesia* when it is detected in a human patient.

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1. Introduction

Babesia are tick-borne parasites in the Phylum Apicomplexa. Two closely related genera, *Theileria* and *Cytauxzoon*, are also tick-borne, and collectively these three genera are referred to as the piroplasms. Many species of piroplasms are significant pathogens and the Apicomplexa also includes numerous other pathogens of veterinary and medical importance including *Plasmodium* spp. (causative agents of malaria), *Cryptosporidium* spp., *Eimeria* spp., *Isospora* spp., and *Toxoplasma gondii*. Several reviews have focused on the clinical aspects of babesiosis in people and domestic animals, the pathogenesis of infection, the phylogenetic relationships of the piroplasms, piroplasm genomics, and their diagnosis and treatment, thus we will review the current knowledge regarding the ecology of *Babesia* among their reservoir and tick hosts with an emphasis of the role of wildlife as reservoirs (Florin-Christensen and Schnittger, 2009; Lau, 2009; Rosenblatt, 2009; Ayoob et al., 2010; Gray et al., 2010; Suarez and Noh, 2011; Lack et al., 2012; Lobo et al., 2012; Matijatko et al., 2012; Mosqueda et al., 2012; Schnittger et al., 2012).

Historically, *Babesia* were classified by two methods, (1) the relative size and shape of trophozoites in the erythrocytes and the number of merozoites and (2) the host of origin. Based on size, there were two groups (small and large *Babesia*), but this division is not associated with phylogenetic relatedness. Identification based on host origin was based on the assumption that these parasites are host-specific, which we now know is not the case for many species. Molecular characterization of multiple gene targets indicates that the piroplasms should be divided into at least five or six groups: one that includes small *Babesia* from various wild rodents, felids, canids, and mesomammals (called archaeopiroplasmids or *Microti* group); one that includes parasites from cervids, dogs, and people (called the western piroplasms, Duncani group or prototheilerids); one that includes primarily canine, bovine, and cervine species (babesids); another that includes primarily bovine, equine, and ovine species (unguilibabesids); and a final group that includes the *Theileria* and *Cytauxzoon* spp. (theilerids) (Criado-Fornelio et al., 2003). Some analyses suggest that the babesids and unguilibabesids are one monophyletic group and that the theilerids represent up to three separate groups. Regardless of divisions, numerous studies support that the organisms currently included in the genus *Babesia* are polyphyletic, and that new genera should be erected to clarify the phylogenetic relationships of the piroplasms (Criado-Fornelio et al., 2003; Lack et al., 2012; Schnittger et al., 2012). Interestingly, zoonotic species are found in all of the groups with *Babesia* spp. but no *Theileria* or *Cytauxzoon* spp. have been identified as zoonotic infections (Fig. 1).

Even at the species level, there is considerable confusion regarding the true number of species. For example, *Babesia microti*, the predominant cause of babesiosis in the United States and a rare cause of disease in people from Europe and Asia, has a holarctic distribution in rodents and insectivores, but recent molecular studies indicate that this species is a species complex that includes at least four 'named' types (US, Munich, Kobe, and Hobetsu (referred to as Otsu in some publications)) and an unknown number of other strains (Goethert et al., 2006; Tsuji et al., 2006; Nakajima et al., 2009). It has been suggested that *B. microti* and other *B. microti*-like species (some erroneously referred to as *Theileria annae*) reported from some rodents and mesomammals (e.g. raccoons, foxes, skunks), *Babesia rodhaini* from rodents, and *Babesia leo* and *Babesia felis* from African felids should collectively be included in a new genus but more research is needed before a change is finalized (Uilenberg, 2006; Nakajima et al., 2009).

Babesiosis in people can range from asymptomatic infections to severe disease and death. Severity of illness depends on many factors, such as *Babesia* species and immunocompetence of the patient. Infections with *B. microti*, the most common species in North America, can result in asymptomatic infection to severe disease which sometimes results in death. Asymptomatic patients are typically diagnosed on routine testing that either identifies the organism in blood smears or during serologic testing (as conducted on some blood donors). It is estimated that about a third of patients remain asymptomatic (Krause et al., 2003). Most patients with *B. microti* infections develop mild to moderate flu-like illnesses characterized by malaise and fatigue that progress to include the following symptoms: rash, fever, chills, sweats, headache, arthralgia, myalgia, anorexia, cough, or nausea. Rarely, mild splenomegaly or hepatomegaly may be noted. Clinical symptoms may persist for weeks to months, but rarely for more than a year. A small percentage of patients (~5–10%), especially those that are immunosuppressed or splenectomized, may develop severe disease from *B. microti* infection. These patients may present with jaundice, or diffuse ecchymoses or petechiae (Rosner et al., 1984).

The most common zoonotic species in Europe, *Babesia divergens*, requires rapid diagnosis as clinical disease is often severe. Generally the parasite has an incubation period of one to three weeks during which the patients may begin to describe malaise and discomfort. Onset of serious illness is sudden, with hemoglobinuria as the most common sign (Telford and Spielman, 1993). Clinical signs of *B. divergens* infection are easily confused with those of malaria including jaundice, non-periodic fever, sweats, shaking, headache, vomiting, diarrhea, etc. (Gorenflot et al., 1998). It is estimated that most cases of *B. divergens* infection resulted in major organ failure and death within four to seven days after the onset of hemoglobinuria (Gorenflot et al., 1987; Kjemtrup and Conrad, 2000). However, prompt therapy with blood transfusion and ventilation has reduced the *B. divergens* mortality rate (Zintl et al., 2003). Recent reviews have discussed the clinical course of infection, diagnosis, and treatment option for babesiosis in detail (Gray et al., 2010; Mosqueda et al., 2012).

2. Natural history of zoonotic *Babesia*

The *Babesia* are one of the most common haemoparasites in the world, second only to trypanosomes, and have a wide host range, including hundreds of mammal species and a limited number of bird species. *Babesia* parasites are maintained in a complex system of tick vectors and animal reservoirs. People are not a natural host for any species of *Babesia* but can serve as accidental hosts for numerous species (Fig. 2, Table 1).

The complete life cycle for a large percentage of *Babesia* species is unknown, but ticks in the family Ixodidae are the only known vectors. One possible exception is *Ornithodorus erraticus*, an argasid soft tick, which may transmit *Babesia meri*, a parasite of sand rats (*Psammomys obesus*) in Africa (Gunders, 1977; René et al., 2012; Ros-García et al., 2011; Schwint et al., 2008; Razmi and Nouroozi,

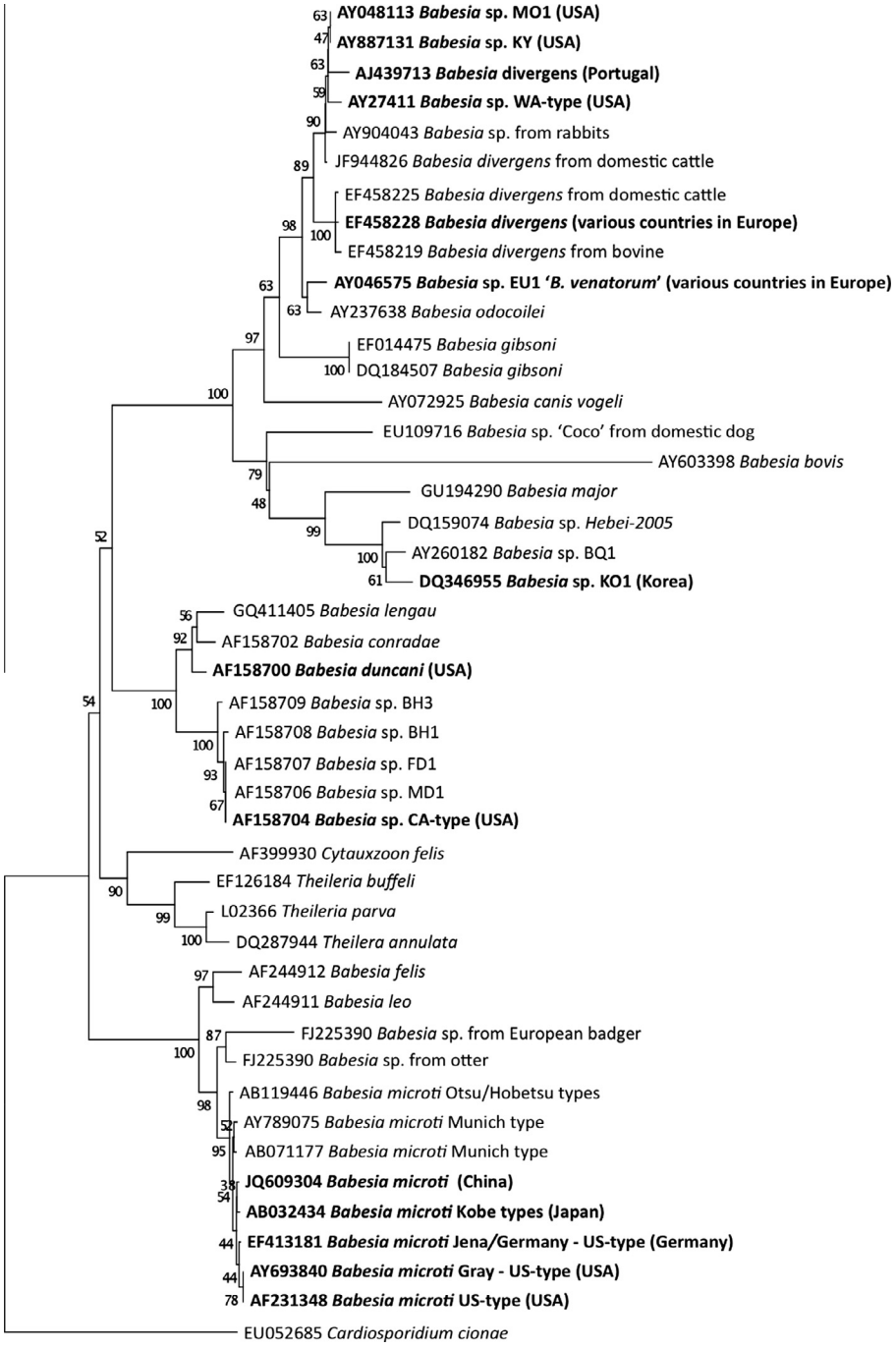

Fig. 1. Phylogenetic tree of *Babesia* and related piroplasms with zoonotic species bolded. The tree was constructed using neighbor-joining analysis of full-length 18S rRNA gene sequences extracted from GenBank (accession numbers listed for each species). For zoonotic representatives, the endemic country or region is listed in parentheses.

2010). Even for those with known life cycles, there are still many unknowns, such as possible roles of alterative vectors or hosts, impacts of host community diversity on prevalence in ticks and hosts, cross-protection among subsequent infections with the same or closely related *Babesia*, ability of ticks to serve as reservoirs in the absence of vertebrate hosts (for those that utilize transovarial transmission), and duration of infectivity of the *Babesia* to tick vectors.

Another potential aspect of *Babesia* transmission that is understudied, and may be important in the ecology of zoonotic infections, is the use of "bridge vectors". Some pathogens can be maintained in nature by one or more vectors that may be very host specific and are unlikely to or only rarely feed on people. If a tick species that will feed on people and/or a competent reservoir host is present or introduced into the sylvatic cycle, the more catholic vector could transmit *Babesia* to people. Goethert et al. (2003) discovered that *B. microti* is maintained in nature by a rodent-associated tick (*Ixodes angustus*) in an area without *Ixodes scapularis*; however, genetic testing revealed that the *B. microti* associated with *I. angustus* was different from zoonotic strains of *B. microti*. Similarly, *B. microti* is maintained in the United Kingdom by another rodent-associated tick (*Ixodes trianguliceps*); however, genetic characterization of these *B. microti* strains has not been conducted (Bown et al., 2008). More research is needed to understand the role of bridge-vectors in the ecology of zoonotic *Babesia* species.

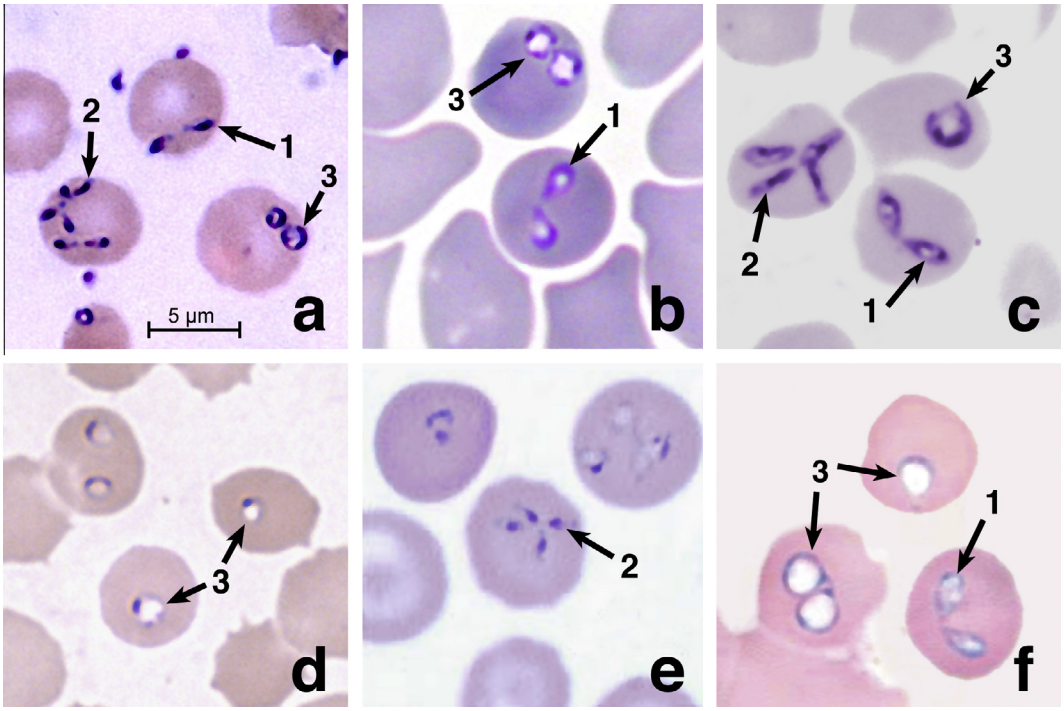

Fig. 2. *Babesia* parasites in human erythrocytes. (a) *B. divergens*, (b) *B. venatorum*, (c) *Babesia* sp. MO1 from Kentucky, (d) *B. microti*, (e) *B. duncani*, (f) *Babesia* sp. KO1 from Korea. (1) Paired piriforms; (2) Tetrads; (3) Ring forms. The figure was reprinted with permission from Elsevier first published in Gray et al. (2010). The parasites shown in Fig. 2a, b, c, e, and f were assembled from original photographs, first published as follows: (a) Hunfeld et al., 2008; (b) Häselbarth et al., 2007; (c) Beattie et al., 2002; (e) Kjemtrup et al., 2002; (f) Kim et al., 2007.

Table 1
Natural history characteristics of the primary agents of human babesiosis.

Species	Geographical distribution	Vector(s)[a]	Reservoir hosts[b]
Babesia microti complex			
Babesia microti	USA (northeast, upper Midwest, and mid-Atlantic)	Ixodes scapularis	Rodents
Babesia sp. Kobe	Asia (Japan, Russia, Korea)	Unknown	Rodents
Babesia sp.	China	Ixodes persulcatus	Rodents
Babesia sp. Munich	Europe	Ixodes ricinus	Rodents
B. microti-like sp.	Australia	Unknown	Rodent?
Babesia sp. TN1	USA (Tennessee)	Unknown	Unknown
Babesia sp. KO1	Korea	Unknown	Unknown
Babesia duncani (= *Babesia* sp. WA1)	USA (California, Washington)	Unknown	Unknown
Babesia sp. CA-type	USA (California)	Unknown	Unknown
Babesia sp. MO1	USA (Missouri, Kentucky, Washington, Massachusetts)	Ixodes dentatus	Lagomorphs
Babesia sp. (*B. divergens*-like)	China	Unknown	Unknown
Babesia sp. (*B. divergens*-like)	Canary Islands, Spain	Unknown	Unknown
Babesia divergens	Europe	Ixodes ricinus	Bovines
Babesia sp. EU1 (= *B. venatorum*)	Europe	Ixodes ricinus	Cervids
Babesia sp.	Mexico	Unknown	Unknown
Babesia spp.	South America (Columbia, Brazil)	Unknown	Unknown
Babesia spp.	Africa (South Africa, Egypt)	Unknown	Unknown

[a] Primary vector(s) to humans. Other tick species may maintain infections among reservoir hosts.
[b] Additional details regarding reservoir host(s) are given in the text.

An alternative route of transmission is through blood transfusion, and in North America babesiosis is the most common transfusion–transmitted infection (Young et al., 2012). Worldwide, little attention has been given to transfusion-associated cases, but they likely occur in areas where babesiosis is endemic (Hildebrandt et al., 2007). Another route of transmission is vertical (from mother to infant); several transplacental cases associated with *B. microti* have been reported in the Northeastern United States (Joseph et al., 2012).

2.1. Babesia in North America

The first case of human babesiosis in North America, was diagnosed in a splenectomized patient from California (USA) in 1966 (Scholtens et al., 1968). The causative agent wasn't identified, but was likely *Babesia duncani* or the *B.* sp. CA-type. Soon after the initial case in California, a case caused by *B. microti* was diagnosed in a patient from Massachusetts (USA) in 1969 (Western et al., 1970). Although *B. microti* is the primary cause of human babesiosis the United States, at least four other species have been associated with human infections including *B. duncani*, *B.* sp. CA-type, *B.* sp. MO1, and newly discovered *Babesia* sp. from a patient in Tennessee (Table 1) (Fig. 2) (Persing et al., 1995; Herwaldt et al., 1996; Conrad et al., 2006; Moncayo et al., unpublished). Tick-transmitted cases of babesiosis have not been reported in Canada, but transfusion-associated transmission of *B. microti* has occurred (Kain et al., 2001). In Mexico, *Babesia* has been isolated from three individuals

by inoculation of splenectomized hamsters, but no molecular characterization was conducted (Osorno et al., 1976).

2.1.1. Babesia microti

2.1.1.1. Humans. The first case of *B. microti* infection in the United States was detected in 1969 in Massachusetts (Western et al., 1970). The epidemiology of human babesiosis in the United States is similar to Lyme disease with the majority of human cases diagnosed in the northeastern and upper Midwestern United States. In January 2011, babesiosis became reportable in 18 states and one city, and during 2011, 1124 confirmed and probable cases were reported from 15 of the 18 states where babesiosis is reportable (Herwaldt et al., 2012). Most (97%) of cases were reported from seven states (Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin) (Herwaldt et al., 2012). Babesiosis has been reported in asplenic and spleen-intact patients, but disease is most severe in immunocompromised patients.

Infections resulting from blood transfusions have been reported and are probably responsible for sporadic cases occurring in non-endemic states (e.g., Texas, California, Washington, and Georgia) and countries (e.g., Canada) (Kain et al., 2001). From 1980 to 2010, it is estimated that 70–100 transfusion–transmitted infections occurred in the United States (Leiby, 2011). Within highly endemic areas (Connecticut, New York, and Massachusetts), seroprevalence among blood-donors ranged between 0% and 4.3% and importantly, over 50% of seropositive patients were PCR positive (Popovsky et al., 1988; Krause et al., 1991; Linden et al., 2000; Leiby et al., 2002, 2005; Johnson et al., 2009, 2012).

2.1.1.2. Reservoirs. In the eastern US, where the incidence of human babesiosis is highest, the primary reservoir is the white-footed mouse (*Peromyscus leucopus*) (Anderson et al., 1991; Telford and Spielman, 1993; Stafford et al., 1999). However, infections with morphologically similar *Babesia* have been reported in other rodents that are sympatric with *P. leucopus* (e.g., meadow voles (*Microtus pennsylvanicus*), short-tailed shrews (*Blarina brevicauda*), brown rats (*Rattus norvegicus*), Eastern cottontail rabbits (*Sylvilagus floridanus*), and Eastern chipmunks (*Tamias striatus*)) (Healy et al., 1976; Spielman et al., 1981; Anderson et al., 1986, 1987; Telford et al., 1990). In general, prevalences in reservoir hosts are high (>25%) (Healy et al., 1976; Spielman et al., 1981; Anderson et al., 1986). A recent study by Hersh et al. (2012), described the reservoir competence for a suite of potential hosts by collecting engorged *I. scapularis* larvae and testing resulting nymphs for *B. microti*. Two strains of *B. microti* were detected in the nymphs, one was a strain associated with human infections, but the other was genetically unique and only found in nymphs from opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and a single wood thrush (*Hylocichia mustelina*). For the *B. microti* strain associated with human infections, the white-footed mouse had the highest reservoir competence (average of 29.5% of ticks became infected) followed by short-tailed shrews and eastern chipmunks (averages of 21.9%, and 17.6%, respectively). Interestingly, masked shrews (*Sorex cinereus*) also infected a high percentage of ticks, but only a limited number of ticks and hosts were tested.

In Maine, where *I. scapularis* is absent or rare, a *B. microti* that was genetically distinct from human-infecting strains was detected in a redback vole (*Clethrionomys gapperi*), a masked shrew (*S. cinereus*), and a short-tailed shrew (Goethert et al., 2003). Interesting data from England and Japan suggest that shrews (*Sorex* spp.) are important hosts; however, few studies have been conducted on US *Sorex* spp. (Burkot et al., 2000; Goethert et al., 2003; Zamoto et al., 2004b; Bown et al., 2011) Many of these *B. microti* reservoirs are also competent reservoirs for two other zoonotic pathogens, *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, so coinfections of reservoirs and ticks are common (Magnarelli et al., 2006; Abrams, 2008; Tokarz et al., 2010). Experimental or field-based studies are needed to better understand the reservoir competence of rodent species for *B. microti* in the Northeastern US.

Infections with *B. microti*, based on either morphology or PCR analysis, have been reported in rodents in the western and southeastern US where *B. microti*-associated human babesiosis is not known to be endemic. Recently, a high prevalence of *B. microti* (genetically similar to human-associated strains) was detected in cotton rats (*Sigmodon hispidus*) in Florida (Clark et al., 2012). In Alaska, *B. microti* (genetically distinct from human-associated strains) has been detected in Northern red-backed voles (*Myodes* (*Clethrionomys*) *rutilus*), tundra voles (*Microtus oeconomus*), singing voles (*Microtus miurus*), shrews (*Sorex* spp.), and Northwestern deer mice (*Peromyscus keeni*) (Goethert et al., 2006). In Colorado, *B. microti* was identified in 13 of 15 prairie voles (*Microtus ochrogaster*) by PCR of blood or spleens (Burkot et al., 2000). Similarly in Montana, nearly half of all montaine voles (*Microtus montanus*), meadow voles, and water voles (*Arvicola richardsoni*) tested by blood or spleen smears were infected with *B. microti*, whereas none of 40 deer mice (*Peromyscus maniculatus*) were infected (Watkins et al., 1991). Uncharacterized *Babesia* spp. have been detected in rodents from Wyoming and California (Wood, 1952; van Peenen and Duncan, 1968; Watkins et al., 1991). *B. microti* from microtine rodents in Alaska are phylogenetically related to strains detected in other rodent species in Montana and Maine, but these parasites were distinct from human-associated *B. microti* strains from the United States, Asia, and Europe (Goethert et al., 2006). Therefore, the finding of *B. microti* (based on morphology) in rodents in a particular geographic area might not suggest a high risk of human infection. As additional evidence that genetic characterization is needed, a small piroplasm from dusky-footed woodrats (*Neotoma fuscipes*) in California was shown to be a *Theileria* species (Kjemtrup et al., 2001).

2.1.1.3. Vectors. In the United States, the primary vector responsible for transmission of *B. microti* to humans is *I. scapularis.* Other rodent-associated *Ixodes* species (e.g., *I. angustus*, *I. eastoni*, *I. muris*, and *I. spinipalpis*) are known or suspected sylvatic vectors of the parasite, or genetically related strains (Watkins et al., 1991; Burkot et al., 2000; Goethert et al., 2003; Tokarz et al., 2010). These other *Ixodes* spp. are primarily nidicolous and are considered low risk for transmission of *B. microti* to people, but rare reports of human infestation have been reported (Anastos, 1947; Damrow et al., 1989; Zeidner et al., 2000). Infection rates for adult *I. scapularis* in the Northeastern and Midwestern United States are typically low (<3%), although rates as high as 30% have been reported (Steiner et al., 2008; Walk et al., 2009; Tokarz et al., 2010). Nymphs and adult *I. scapularis* can transmit *B. microti* to humans but transmission takes at least 48 hours of feeding, so prompt removal of ticks can prevent transmission (Piesman and Spielman, 1980; Johnson et al., 2009). Transovarial transmission has not been proven for *B. microti*, (Oliveira and Kreier, 1979; Walter and Weber, 1981), but results from Hersh et al. (2012) suggest it may occur for some strains as *Babesia* positive nymphs resulted from larvae that engorged on opossums and passerine birds which are not known to be hosts for *B. microti*.

2.1.2. Babesia duncani

A *Babesia* sp. (originally referred to as *Babesia* sp. WA1), morphologically indistinguishable from *B. microti*, was recognized in babesiosis patients from Washington and California in the early 1990s (Quick et al., 1993; Thomford et al., 1994). This parasite has been formally named *B. duncani* (Conrad et al., 2006). Although morphologically similar parasites have been reported from people

in California previously, it is unknown if these were *B. duncani* or *B.* sp. CA type (Scholtens et al., 1968; Bredt et al., 1981).

Importantly, cases have been documented in immunocompetent individuals with spleens as well as immunocompromised individuals and blood transfusion-acquired cases have been reported (Herwaldt et al., 2011; Bloch et al., 2012). Serological studies conducted near cases in California and Washington indicated that 3.5–16% of individuals were seropositive (Quick et al., 1993; Persing et al., 1995). Even higher seroprevalences have been reported among blood donors in several states outside the endemic range, which is likely due to movement of infected individuals or infection with one or more *Babesia* spp. that cross-react with *B. duncani* (Prince et al., 2010).

Phylogenetic analysis of *B. duncani* indicated that it is in a separate clade from other *Babesia* species that includes *B. conradae* from dogs in California, a *Babesia* sp. from woodrats in Texas, *B. lengau* from cheetahs in Africa, and *B. poelea* and *B. uriae* from seabirds (Fig. 1) (Kjemtrup et al., 2000; Yabsley et al., 2005, 2006a, 2009; Conrad et al., 2006; Bosman et al., 2010; Charles et al., 2012). This group was also related to *Babesia* sp. CA-type from humans in California and other *Babesia* from mule deer (*Odocoileus hemionus*) and bighorn sheep (*Ovis canadensis*) in California (Kjemtrup et al., 2000), Significantly, to date, neither a reservoir nor a tick vector has been identified. Testing of numerous rodent, insectivore, and lagomorph species in Washington was uniformly negative for *B. duncani* (Quick et al., 1993; Persing et al., 1995).

2.1.3. Babesia sp. CA-type

Four severe to fatal human cases with *Babesia* sp. CA-type have been reported in splenectomized patients from California (Persing et al., 1995). This species is morphologically indistinguishable from *B. duncani*, but can be distinguished by genetic sequencing of multiple targets. No vector or reservoir has been identified, but genetically similar organisms have been detected in mule deer, bighorn sheep, and a captive fallow deer (*Dama dama*) from California. However, sequence analysis of the 18S rRNA gene and internal transcribed spacer (ITS)-2 region has revealed limited nucleotide differences between the *Babesia* spp. from cervids and *Babesia* sp. CA-type from humans (Conrad et al., 2006).

2.1.4. Babesia sp. MO1

Babesia sp. MO1 is a rare cause of babesiosis in the United States. In 1992, this novel *Babesia* species was detected in a splenectomized patient in Missouri (Herwaldt et al., 1996). Despite treatment (quinine and clindamycin), the patient died 13 days after diagnosis. Two additional cases of *Babesia* sp. MO1 in splenectomized patients, both nonfatal, have been reported in Kentucky and Washington (Beattie et al., 2002; Herwaldt et al., 2004). The natural hosts of this *Babesia* sp. appear to be lagomorphs. In Massachusetts, 16% of eastern cottontail rabbits (*S. floridanus*) were positive (Goethert and Telford, 2003) and related *Babesia* sequences were subsequently found in desert cottontails (*Sylvilagus audubonii*) and black-tailed jackrabbits (*Lepus californicus*) from Texas (Yabsley et al., 2006b). *Ixodes dentatus* is a competent vector and transmission to rabbits is temporally correlated with peak *I. dentatus* larvae activity (Goethert and Telford, 2003). *I. dentatus* is a common tick on rabbits and birds and it will feed on people, although rarely (Harrison et al., 1997; Hamer et al., 2011).

Initial phylogenetic analyses on a fragment of the 18S rRNA gene indicated that the *Babesia* sp. (MO1) was closely related to *B. divergens*, leading to concern that this European parasite had been introduced into the United States. Subsequent analyses proved that *Babesia* sp. MO1 is distinct from *B. divergens* from Europe based on the additional sequence analysis, lack of infectiousness to cattle, distinct morphology when grown *in vitro*, and erythrocyte *in vitro* specificity (Holman et al., 2005; Holman, 2006).

2.2. Babesia in Europe and Asia

The first human babesiosis case was reported in Europe and occured in 1957 (Skrabalo and Deanovic, 1957). The most common cause of human babesiosis in Europe is *B. divergens*, but other zoonotic species include a *B. divergens*-like species from the Canary Islands, *Babesia* sp. EU1 (also called *B. venatorum*), and a *B. microti*-like species (Fig. 2) (Table 1). As with *Babesia* species in the US, some infections go undiagnosed as demonstrated by a serologic study conducted in Germany where 3.6% and 5.4% of 467 asymptomatic individuals had antibodies reactive to *B. divergens* and *B. microti*, respectively (Hunfeld et al., 2002).

At least four species or genotypes of zoonotic *Babesia* have been detected in Asia with rare infections reported from Japan, Taiwan, Korea, India, and China (Table 1) (Wei et al., 2001; Arai et al., 2003; Marathe et al., 2005; Kim et al., 2007). Cases from Japan, Taiwan, and China have been caused by *B. microti*-like species (Wei et al., 2001; Arai et al., 2003; Kim et al., 2007) while the Korean cases were caused by a novel *Babesia* sp. (KO1) which is related to *B. ovis* (Kim et al., 2007).

2.2.1. Babesia divergens

The first human babesiosis case was caused by *B. divergens* and it occurred in Croatia (Skrabalo and Deanovic, 1957). Human cases are typically severe, especially in splenectomized individuals. To date, approximately 40 cases have been reported, primarily from France, Ireland, and Great Britain with fewer cases reported from Sweden, Switzerland, Spain, Portugal, and Croatia (Centeno-Lima et al., 2003; Moreno Giménez et al., 2006; Martinot et al., 2011). However, undiagnosed exposures do occur, as a seroprevalence of 13% was detected among Lyme disease patients in Sweden (Uhnoo et al., 1992).

Cattle are the natural host for *B. divergens* and infections are noted throughout Europe and possibly into North Africa (Tunisia), which corresponds with the distribution of the only known vector, *Ixodes ricinus* (Zintl et al., 2003). Although cattle are the principal host, infections may have been detected in farmed reindeer (*Rangifer tarandus*) in the United Kingdom; however, these infections may have been caused by *Babesia capreoli* (Malandrin et al., 2010). Extensive molecular or biological characterizations of "*B. divergens*" samples from cervids have revealed that they are distinct and likely are *B. capreoli* (Adam et al., 1976; Schmid et al., 2008; Bastian et al., 2012). In addition, *B. capreoli*, unlike *B. divergens*, lacks infectivity for gerbils and splenectomized cattle (Malandrin et al., 2010). Additional studies are needed to confirm the ability of *B. divergens* to utilize cervids (non-splenectomized) as reservoirs (Zintl et al., 2011).

Experimental *B. divergens* infections have been established in a variety of splenectomized animals including chimpanzees (*Pan troglodytes*), rhesus macaque (*Macaca mulatta*), laboratory rats, roe deer (*Capreolus capreolus*), fallow deer, red deer (*Cervus elaphus*), European mouflon (*Ovis orientalis musimon*), and domestic sheep (Malandrin et al., 2010).

Babesia divergens shares the same vector as *B. capreoli* and two other zoonotic *Babesia* in Europe (*B.* sp. EU1 and *B. microti*). Infections have been reported in *I. ricinus* from Hungary, Austria, Belgium, Netherlands, Switzerland, Germany, Norway, and Estonia (Blaschitz et al., 2008; Wielinga et al., 2009; Schorn et al., 2011; Egyed et al., 2012 Lempereur et al., 2012; Oines et al., 2012). Importantly, surveys of ticks utilizing highly conserved or short regions of the 18S rRNA gene may lead to misidentification of *B. capreoli* and other *B. divergens*-like sp. as *B. divergens*. Transovarial transmission by *I. ricinus* has been documented (Bonnet et al., 2007a).

2.2.2. Babesia divergens-like spp.

A *B. divergens*-like species has been reported in a single patient from the Canary Islands, Spain, in 1994, who had an unidentified

tick attached prior to his illness (Olmeda et al., 1997). Sequence analysis of a short region of 18S rRNA indicated the agent was related to *B. divergens*, but neither *B. divergens* nor its vector (*I. ricinus*) is present on the Canary Islands. Interestingly, it was noted that the patient carried a tick-infested rabbit before becoming sick. Although none of the ticks were available for identification, *Ixodes ventalloi* is a common rabbit-associated tick on the island. Additional work is needed to determine if this species is a variant of *B. divergens* or is related to *B. divergens*-like species that are associated with lagomorphs in the United States.

Recently, two cases of *B. divergens*-like sp. were detected among 377 anemic patients in Shandong Province, China (Qi et al., 2011). Similar to the Canary Islands, neither *B. divergens* nor its vector (*I. ricinus*) is present in China. Currently, no vector or reservoir host is known for this Chinese babesiosis agent.

2.2.3. Babesia sp. EU1

Babesia sp. EU1 was first recognized as a human pathogen in 1998 and was given the designation EU (European Union)-1 because the three initial cases occurred in asplenic patients from Italy and Austria (Herwaldt et al., 2003). An additional case in an immunosuppressed patient from Germany has been reported. The infections ranged from mild to moderately severe, but none were fatal (Herwaldt et al., 2003; Häselbarth et al., 2007). The name *B. venatorum* has been proposed, but a formal description has not been published (Häselbarth et al., 2007).

Roe deer are the natural host of this parasite and infected deer have been reported from Slovenia, France and Italy (Duh et al., 2005a; Bonnet et al., 2007b; Tampieri et al., 2008; Bastian et al., 2012). Prevalences in deer are generally high (>20%) (Duh et al., 2005a; Bonnet et al., 2007b). No infections have been reported in sympatric red deer, although red deer are infected with *B. capreoli* (Duh et al., 2005a). Disease due to *B.* sp. EU1 has been reported for a captive reindeer (*R. tarandus*) in a zoo in The Netherlands (Kik et al., 2011).

The only known vector of *B.* sp. EU1 is *I. ricinus*, and similar to *B. divergens*, infected ticks have been reported throughout Europe including Estonia, Switzerland, Poland, Italy, Belgium, Germany, France, Netherlands, Norway, and Slovenia (Duh et al., 2005b; Casati et al., 2006; Schmid et al., 2008; Cieniuch et al., 2009; Wielinga et al., 2009; Cassini et al., 2010; Burri et al., 2011; Gigandet et al., 2011; Katargina et al., 2011; Reis et al., 2011; Schorn et al., 2011; Capelli et al., 2012; Lempereur et al., 2012; Oines et al., 2012). Transovarial and transstadial transmission of *B.* sp. EU1 by *I. ricinus* has been documented (Bonnet et al., 2007b, 2009; Mazyad et al., 2010), but in general, prevalences in *I. ricinus* are low (<2%) (Cieniuch et al., 2009; Cassini et al., 2010; Katargina et al., 2011; Oines et al., 2012).

Interestingly, this *Babesia* has been detected in *I. ricinus* removed from passerines that migrated to Norway and northwestern Russia, suggesting a risk for establishment in countries currently north of the known distribution (Hasle et al., 2011; Movila et al., 2011). A *Babesia*, closely related or identical to *B.* sp. EU1 have been detected in *Ixodes persulcatus* from the Novosibirsk region of Russia (Rar et al., 2011), but a survey of this tick species in Estonia failed to detect *B.* sp. EU1 (Katargina et al., 2011).

2.2.4. Babesia microti and related species

2.2.4.1. Humans. Currently, only a single case of *B. microti*-associated babesiosis has been confirmed in Europe: a German patient with leukemia who likely became infected by a transfusion (Hildebrandt et al., 2007). Retrospective screening of blood donors for the patient revealed a single donor with a titer to *B. microti*. Neither person had travel history to North America or Asia. Several surveys have detected anti-*B. microti* antibodies in individuals in Croatia and Poland suggesting that infections are underdiagnosed (Topolovec et al., 2003; Chmielewska-Badora et al., 2012).

In Asia, human cases with *B. microti*-like sp. are rare and sporadic infections have been reported from Japan, Taiwan, China, and possibly India (Wei et al., 2001; Arai et al., 2003; Marathe et al., 2005). The first human case (*B. microti* Kobe type) in Japan was diagnosed in a patient in 1999. This patient likely acquired the infection from an asymptomatic donor (Matsui et al., 2000; Wei et al., 2001). Previously diagnosed cases have been reported in Tawain (asymptomatic) (Shih et al., 1997) and in China (Li and Meng, 1984), but the causative agents were not well characterized. Serologic studies in Asia have indicated that unrecognized infections have occurred. In Taiwan, individuals with antibodies to *B. microti* have been reported (Hsu and Cross, 1977; Shih et al., 1997) and a retrospective survey of sera collected in 1985 from Japan indicated that 1.3% of 1335 samples had antibodies to *B. microti* Kobe type ($n = 3$) and *B. microti* Hobetsu type ($n = 14$), with the latter type having only been previously detected in rodents (Tsuji et al., 2001; Arai et al., 2003). Outside of Southeast Asia, antibodies to *B. microti* have been detected in 6% of 273 individuals from northern Turkey (Poyraz and Güneş, 2010).

2.2.4.2. Reservoirs. In Europe, natural infections of *B. microti* have been reported from numerous rodent and shrew species including species of yellow-necked mice (*Apodemus flavicollis*), wood mice (*Apodemus sylvaticus*), bank voles (*Myodes (Clethrionomys) glareolus*), field voles (*Microtus agrestis*), common shrews (*Sorex araneus*), and *Mus* spp. in Germany, Poland, Croatia, Slovenia, Austria, Hungary, Bulgaria, Czech Republic, Slovakia, and the United Kingdom (Sebek et al., 1977, 1980; Turner, 1986; Randolph, 1995; Bajer et al., 2001; Duh et al., 2003; Siński et al., 2006; Beck et al., 2011; Bown et al., 2011). Genetic characterization of various samples of *B. microti* has indicated that both zoonotic and presumed non-zoonotic strains are co-circulating in the same species of rodents (Beck et al., 2011).

In Asia, at least three named types of *B. microti* parasites (US, Kobe, and Hobetsu) have been detected in naturally infected rodents and shrews (Zamoto et al., 2004a,b; Kim et al., 2007; Qi et al., 2011). In Japan, two field mice species (Large Japanese field mice (*Apodemus speciosus*) and Small Japanese field mice (*A. argenteus*)) are natural hosts for *B. microti* Kobe type (Shiota et al., 1984; Tsuji et al., 2001; Wei et al., 2001; Saito-Ito et al., 2004, 2007). Numerous rodents and shrews are infected with *B. microti* Hobetsu type including Large Japanese field mice, grey red-backed voles (*Clethrionomys rufocanus*), northern red-backed voles (*C. rutilus*), Japanese field voles (*Microtus montebelli*), long-clawed shrews (*Sorex unguiculatus*), and Laxmann's shrews (*Sorex caecutiens*) (Tsuji et al., 2001). Large Japanese field mice, grey red-backed voles, and northern red-backed voles are also hosts for *B. microti* US-like (Zamoto et al., 2004a,b). In Taiwan and China, *B. microti* Kobe type or related parasites have been reported in Horsfield's shrews (*Crocidura horsfieldii*) and spinous country-rats (*Rattus coxinga*), Chinese white-bellied rats (*Niviventer confucianus*) and striped field mice (*Apodemus agrarius*) (Saito-Ito et al., 2008). *B. microti* US type has been found in striped field mice and Korean field mice (*Apodemus peninsulae*) from South Korea, yellow steppe lemmings (*Eolagurus* (= *Lagurus*) *luteus*) from China, and Korean field mice and grey red-backed voles from Eastern Russia (Zamoto et al., 2004b). A related *B. microti*-like sp. has been detected in Eurasian red squirrels (*Sciuris vulgaris*) (Tsuji et al., 2006).

2.2.4.3. Vectors. In Europe, the primary vector of *B. microti* is *I. ricinus*, which also transmits *B. divergens* and several other human and veterinary pathogens (e.g., *Borrelia* and *Babesia*). This tick is common on large mammals, including people, throughout Europe and isolated parts of western Asia and northern Africa. Infections

with *B. microti*-like species have been reported in *I. ricinus* throughout the range of the tick including Switzerland, Poland, Italy, the Netherlands, Czech Republic, Estonia, Belgium, Hungary, Germany, Russia, and the United Kingdom (Alekseev et al., 2003; Hartelt et al., 2004; Rudolf et al., 2005; Sréter et al., 2005; Casati et al., 2006; Siński et al., 2006; Nijhof et al., 2007; Bown et al., 2008; Wielinga et al., 2009; Cassini et al., 2010; Burri et al., 2011; Gigandet et al., 2011; Katargina et al., 2011; Lempereur et al., 2011).

In England, both *I. ricinus* and *I. trianguliceps* can transmit *B. microti*-like spp. among voles but *I. trianguliceps* is believed to be the primary vector because exclusion of deer (with subsequent drop in numbers of *I. ricinus*) did not affect density of *I. trianguliceps* or prevalence of *Babesia* in voles (Bown et al., 2008). Naturally infected *I. trianguliceps* have also been reported in Poland and Russia (Telford et al., 2002; Karbowiak, 2004). Interestingly, *B. microti* was recently detected in 4.5% of 468 questing *Dermacentor reticulatus* from Poland, suggesting a need to investigate other potential vectors (Wójcik-Fatla et al., 2012).

In Asia, *B. microti* Hobetsu have been detected in questing *Ixodes ovatus* from Japan while *B. microti* US type and a *B. microti* type related to *B. microti* Kobe type have been detected in *I. persulcatus* from Russia and China (Saito-Ito et al., 2004; Yano et al., 2005; Sun et al., 2008; Rar et al., 2011; Zamoto-Niikura et al., 2012). In Taiwan, *Ixodes granulatus* transmitted a *B. microti* strain to laboratory rats (van Peenen et al., 1977).

2.2.5. Babesia sp. KO1

The first human case of babesiosis in Korea was diagnosed in a splenectomized patient from Jeon-nam Province (Fig. 2) (Kim et al., 2007). She was successfully treated with clindamycin after treatment with quinine, for her initial diagnosis of malaria, failed. Parasites observed in her blood were classified as a larger *Babesia* and molecular characterization indicated it was related to *Babesia* sp. from sheep in China. A subsequent PCR-based survey of 68 residents from the patient's village detected three asymptomatic cases (Kim et al., 2007). All goats tested from the village were negative and no reservoir is currently known. No vector has been identified for *B.* sp. KO1 but *Haemaphysalis longicornis* and *Haemaphysalis quinghaiensis* are vectors of two related *Babesia* spp. from China (Bai et al., 2002; Guan et al., 2010).

2.3. Babesia in Africa

Human babesiosis cases in Africa are rarely diagnosed; however, widespread occurrence of malaria likely causes an underestimation of the number of babesiosis cases (Table 1). To date, three cases of babesiosis have been reported from Egypt. The first case involved a splenectomized farm worker who became ill while a study on livestock babesiosis was occurring. Serology indicated an active infection and the patient recovered after treatment (Michael et al., 1987). The other cases resulted in mild illnessess that resolved after treatment (El-Bahnasawy and Morsy, 2008; El-Bahnasawy et al., 2011). Two suspected cases in South Africa had intraerythrocytic parasites morphologically consistent with *Babesia*. One patient recovered following treatment with quinine and tetracycline and the other patient died despite treatment (Bush et al., 1990). Antibodies to *Babesia* have been detected in individuals in Nigeria but analysis of blood smears and inoculation of splenectomized calves and rats failed to confirm infection (Leeflang et al., 1976).

Several *Babesia* spp. have been reported from wild and domestic animals in Africa, but because no sequence analysis has been conducted on human cases, no reservoirs for zoonotic *Babesia* spp. are currently known. Similarly, few studies have investigated potential vectors of zoonotic *Babesia* in Africa. A recent study on the Sinai Peninsula (in the Asian portion of Egypt), reported *B. divergens*-like sp. and *B.* sp. EU1 infections in *I. ricinus* (Mazyad et al., 2010).

2.4. Babesia in Australia

The first case of babesiosis in Australia was diagnosed in 2010 (Table 1) (Senanayake et al., 2012). The fatal case occurred in a 56-year-old man with several previous medical issues. Ovoid forms and tetrads consistent with *Babesia* were found on blood smear and he had a positive antibody titer for *B. microti*. The complete 18S rRNA gene sequence was 100% similar to *B. microti* and partial β-tubulin gene sequence was similar to strains of *B. microti* from the United States. The patient reported previous tick bites and only had a travel history to New Zealand (40 years previously). Following this report, a patient with no travel history outside of Australia tested positive for antibodies reactive with *B. microti* and *B. duncani*, and an additional patient with no travel history outside of Australia tested positive for antibodies to *B. duncani* (Mayne, 2011). Although *Babesia* spp. have been reported from domestic and wild animals in Australia, there is currently no reservoir nor vector identified for this zoonotic *Babesia* sp.

2.5. Babesia in South America

Only three cases of uncharacterized babesiosis have been reported from South America, two from Brazil and one from Columbia (Table 1) (Ríos et al., 2003; Rech et al., 2004). However, serologic studies indicate that exposure occurs commonly in some regions and that infections are either asymptomatic or not diagnosed (Alecrim et al., 1983; Rech et al., 2004; Ríos et al., 2003). Sera from two borreliosis cases in Brazil and one from Columbia reacted with *B. bovis* antigens (Ríos et al., 2003; Yoshinari et al., 2003) and *B. bovis* antibodies have also been detected in 10% of 49 individuals and 25% of 59 Lyme borreliosis patients in Brazil (Yoshinari et al., 2003). Antibodies to *B. microti* have been detected in 31% of 80 individuals in Columbia (Buelvas et al., 2008). However, the causative agents are unknown because the serologic assays used crude antigens which can result in cross-reactions.

3. Detection and characterization methods

The primary method to diagnose babesiosis is the observation of parasites within erythrocytes on Giemsa or Wright's stained thin blood smears. Parasites can also be visualized by UV illumination after staining with acridine orange. Acute cases of babesiosis are typically easily diagnosed by this method because high numbers of parasites are often observed; however, in chronic cases or when trying to diagnose infection in a nonclinical reservoir, this method lacks sensitivity. In addition, few morphological characters are useful in distinguishing *Babesia* species. The most common criterion is size, but even among the 'small' (typically 1–2.5 μm) or 'large' *Babesia* (typically 3–5 μm), most species are morphologically indistinguishable, thus molecular methods are important for species identification (Kjemtrup et al., 2000; Birkenheuer et al., 2004; Conrad et al., 2006). Importantly, the morphology of a particular *Babesia* species may vary, especially if in an aberrant host, so morphology alone cannot be used to definitively identity a parasite to species (Demeter et al., 2011).

Serologic testing is commonly used to diagnose infections when homologous antigens, or antigens from a very closely related species, are available. Because not all human infections result in clinical disease, these chronic cases are more likely diagnosed by serology, rather than blood smear, because few parasites are found in the peripheral blood. In the United States, serologic tests for *B. microti* are considered sensitive and specific because the other

Babesia that infect humans in the United States (i.e., *B. duncani*, *B.* sp. CA-type, and *Babesia* sp. MO1) have limited reactivity with *B. microti* antigens (Quick et al., 1993; Herwaldt et al., 1996). But, as novel *Babesia* are discovered, serologic cross-reactivity may be observed. Also, as noted, some reports of human *Babesia* infection is only based on serology, which cannot be use to definitively identify the *Babesia* species. Sera from *B. duncani* patients reacted with the closely related *B. conradae* (also called "California isolate" of *Babesia gibsoni*), but only minimally with *B. microti* (Quick et al., 1993). Similarly, sera from the *Babesia* sp. MO1 patient only minimally reacted with *B. duncani* and *B. microti* antigens but strongly reacted with *B. divergens* antigens (Herwaldt et al., 1996). In Europe, serologic testing is rarely used to diagnose *B. divergens* infections because of the rapid course of illness (Gorenflot et al., 1998). Serum samples from patients infected with *Babesia* sp. EU1 reacted with *B. divergens* but not *B. microti* or *B. duncani* (Herwaldt et al., 2003). *B. microti* Hobetsu and Kobe types exhibit limited reactivity with *B. microti* US type from the United States as only three of 18 sera positive for high *Babesia* Hobetsu or Kobe type titers reacted with *B. microti* US type (Arai et al., 2003).

Inoculation of laboratory animals can be used to diagnose infection with some *Babesia* species (e.g., *B. microti* and *B. duncani*, which both readily grow in hamsters (*Mesocricetus auratus*) and Mongolian gerbils (jirds) (*Meriones unguiculatus*)) (Gleason et al., 1970; Quick et al., 1993). *B. duncani* can also infect laboratory mice (Hemmer et al., 2000), and an inoculated, splenectomized domestic dog seroconverted, but parasites were not observed in blood samples (Thomford et al., 1994). *Babesia* sp. MO1 does not replicate in laboratory hamsters or Mongolian gerbils nor in splenectomized calves or bighorn sheep (Herwaldt et al., 1996, 2004). Although *B. divergens* can infect a wide range of ungulates, including its natural host (cattle) and immunosuppressed experimental hosts (e.g.,

Table 2
Selected polymerase chain reaction (PCR) assays developed for *Babesia* that can be used for detection and molecular characterization.

Target gene	Primer name	Primer sequence (5′–3′)	References
18S rRNA of *Babesia* spp.	bab1	CTTAGTATAAGCTTTTATACAGC	Persing et al. (1992)
	bab4	ATAGGTCAGAAACTTGAATGATACA	Persing et al. (1992)
	bab2	GTTATAGTTTATTTGATGTTCGTTT	Persing et al. (1992)
	bab3	AAGCCATGCGATTCGCTAAT	Persing et al. (1992)
	PiroA	AATACCCAATCCTGACACAGGG	Armstrong et al. (1998)
	PiroB	TTAAATACGAATGCCCCCAAC	Armstrong et al. (1998)
	CryptoF	AACCTGGTTGATCCTGCCAGT	Duh et al. (2005a)
	CryptoR	GCTTGATCCTTCTGCAGGTTCACCTAC	Duh et al. (2005a)
	3.1	CTCCTTCCTTTAAGTGATAAG	Yabsley et al. (2005)
	5.1	CCTGGTTGATCCTGCCAGTAGT	Yabsley et al. (2005)
	RLBH-F	GAGGTAGTGACAAGAAATAACAATA	Schouls et al.(1999)
	RLBH-R	TCTTCGATCCCCTAACTTTC	Schouls et al. (1999)
	Babfor	GACTAGGGATTGGAGGTC	Blaschitz et al. (2008)
	Babrev	GAATAATTCACCGGATCACTC	Blaschitz et al. (2008)
	BdiF	CAGCTTGACGGTAGGGTATTGG	Oines et al. (2012)
	BdiR	TCGAACCCTAATTCCCCGTTA	Oines et al. (2012)
	TaqMan probe BdiT	FAM-CGAGGCAGCAACGG-MGB	Oines et al. (2012)
ITS1 of *Babesia* spp.	15C	CGATCGAGTGATCCGGTGAATTA	Bostrom et al. (2008)
	13B	GCTGCGTCCTTCATCGTTGTG	Bostrom et al. (2008)
	15D	AAGGAAGGAGAAGTCGTAACAAGG	Bostrom et al. (2008)
	13C	TTGTGTGAGCCAAGACATCCA	Bostrom et al. (2008)
	ITS1for	CGAGTGATCCGGTGAATTATTC	Blaschitz et al. (2008)
	ITS1rev	CCTTCATCGTTGTGTGAGCC	Blaschitz et al. (2008)
ITS2 of *Babesia* spp.	For7	AGCCAATGCGATAAGCATT	Shock et al. (2012)
	Rev7	TCACTCGCCGTTACTAGGAGA	Shock et al. (2012)
	ITS2for	GGCTCACACAACGATGAAGG	Blaschitz et al. (2008)
	ITS2rev	CTCGCCGTTACTAAGGGAATC	Blaschitz et al. (2008)
ITS1, 5.8S, and ITS2 of *Babesia* spp.	1055F	GGTGGTGCATGGCCG	Holman et al. (2003)
	ITSR	GGTCCGTGTTTCAAGACGG	Holman et al. (2003)
	1200F	CAGGTCTGTGATGCT	Holman et al. (2003)
	ITSF	GAGAAGTCGTAACAAGGTTTCCG	Holman et al. (2003)
	LSUR300	TWGCGCTTCAATCCC	Holman et al. (2003)
β-Tubulin of *Babesia* spp.	F34	TGTGGTAACCAGATYGGWGCCAA	Caccio et al. (2000)
	R323	TCNGTRTARTGNCCYTTRGCCCA	Caccio et al. (2000)
	F79	GARCAYGGNATNGAYCCNGTAA	Caccio et al. (2000)
	R206	ACDGARTCCATGGTDCCNGGYT	Caccio et al. (2000)
	Tubu63F	CAAATWGGYGCMAARTTYTGGGA	Zamoto et al. (2004a)
	Tubu-3'	TCGTCCATACCTTCWCCSGTRTACCAGTG	Zamoto et al. (2004a)
β-Tubulin of *Babesia microti*	B-tub-F1	GTTAGATCTGGYCCATACGG	Clark et al. (2012)
	B-tub-R1	TGTATTGTTGTGARCCACGGC	Clark et al. (2012)
CCT7 of *B. microti*	CCT-390F	GATTTCATAATGGARGGMATGGCDCCTCAGA	Nakajima et al. (2009)
	CCT-1327R	TCYCTYARTATDCGTGATATTTCCATCT	Nakajima et al. (2009)
CCT7 of *Babesia* spp.	CCT-262F	CARGATGAYGARGTKGGDGATGGWACBAC	Nakajima et al. (2009)
	TBcct- 3'R1	GGCASGCKGCYTCAGTSGCTGMGTA	Nakajima et al. (2009)
	TBcct35F	TGAAGGARGGMACNGAYACWTCYCARGG	Nakajima et al. (2009)
	TBcct1519R	GTYTTYTTHACBAGGCTGGGCTCCCADA TRCA	Nakajima et al. (2009)
HSP70 of *Babesia* spp.	HSP70for	GCTATTGGTATTGACTTGGG	Blaschitz et al. (2008)
	HSP70rev	CCTTCATCTTGATAAGGACC	Blaschitz et al. (2008)
HSP70 of *Babesia* spp.	Various	Various	Yamasaki et al. (2007)

mouflon, red deer, roe deer, fallow deer, and reindeer) (Malandrin et al., 2010), the only laboratory animal that was susceptible to infection was the Mongolian gerbil (Lewis and Williams, 1979). *Babesia* sp. EU1 did not infect Mongolian gerbils (Herwaldt et al., 2003). Japanese strains of *B. microti*-like sp. also infect hamsters (Shih et al., 1997; Tabara et al., 2007). Additionally, considerable progress has been made in the epidemiology of zoonotic *Babesia* from Japan by detecting *Babesia* parasites in wild rodent reservoirs by inoculating SCID mice whose erythrocytes have been replaced with human erythrocytes (Saito-Ito et al., 2000; Tsuji et al., 2001).

Although *Babesia* species were historically identified on morphologic characteristics alone (e.g., size, general morphology, formation of tetrads), it is now recognized that additional data such as life history (e.g., vector), serologic cross-reaction, and host-specificity are needed to definitively identify *Babesia*. Because these data are lacking for many species, molecular techniques have become particularly important in the diagnosis and classification of *Babesia* species (Table 2). Chronic mild infections or those recently acquired are more likely to be diagnosed by PCR than by blood smear analysis or serology, respectively, because PCR is more sensitive for detecting low numbers of circulating parasites, or in the case of early acute infections, before seroconversion occurs (Schwint et al., 2009). Importantly, PCR can be useful in the diagnosis of babesiosis in areas where malaria is endemic because *Babesia* may look similar to some stages of *Plasmodium* (Persing et al., 1992).

PCR assays targeting several genetic targets have been developed for the detection and characterization of *Babesia* (Table 2). Some assays are highly specific for certain species, which is useful for diagnosis; however, genus-wide PCR assays and subsequent DNA sequencing are also useful for the detection of *Babesia*, as novel species may be detected or unexpected species may be present. For example, a patient from California, where only *B. duncani* and *Babesia* sp. CA-type have been associated with human infections, was diagnosed with *B. microti* which was acquired from blood products from a donor from the northeastern United States (Ngo and Civen, 2009).

4. Conclusions and future perspectives

Because babesiosis of humans is exclusively a zoonosis, it is imperative that we understand the natural history (vertebrate and tick hosts) for the various *Babesia* species that infect people. Some zoonotic *Babesia* (e.g., *B. divergens*) are associated primarily with domestic animals, but most zoonotic *Babesia* are maintained in wildlife reservoirs. The incidence and diversity of tick-borne infections have increased in recent years because of better diagnostic tools, increased awareness, increased contact with natural areas and vectors (habitat encroachment), increased number of immunosuppressed and/or splenectomized individuals, and changes in the environment, for example sub urbanization, which have led to increased densities of ticks and potential reservoir hosts.

For example, the incidence and geographic range of Lyme disease, caused by *B. burgdorferi*, is increasing in North America and Europe (Bacon et al., 2008; Rizzoli et al., 2011). In the United States, *B. microti* and *B. burgdorferi* utilize the same vector and reservoir, and in Europe, *B. divergens* and *B. burgdorferi* utilize the same vector. Thus, measures to control and prevent Lyme disease may affect the incidence of *Babesia* infections. Another factor that may impact incidence of babesiosis is global climate change which may cause a shift in the distribution and density of Ixodid ticks (Gilbert, 2010; Jaenson et al., 2012; Estrada-Peña et al., 2012). As with other vector-borne pathogens with vertebrate reservoirs, determining the effects of climate change on the pathogen or vector is complicated because climate changes may also cause changes in the distribution or density of the vertebrate reservoir hosts (Schloss et al., 2012).

For many cases of babesiosis, the causative agents are unknown and for many agents of babesiosis, the reservoir(s) and/or vectors are unknown (Table 1). While these data are critical to our understanding of these zoonoses, understanding the epidemiology of these parasites is not as simple as knowing the reservoir hosts and vectors. Ixodid tick vectors may primarily depend on non-reservoir hosts for blood meals (e.g., cervids) that are not reservoirs for some *Babesia* such as *B. microti* or *B. divergens* but are important hosts for the vectors such as *I. scapularis* and *I. ricinus*, respectively (Paddock and Yabsley, 2007; Gilbert et al., 2012). Recent data also suggest that mesopredator populations may be important factors in the ecology of *B. microti* in the US. For example, red foxes (*Vulpes vulpes*) are predators of *B. microti* reservoirs, but fox numbers are decreasing in many areas due to the expansion of coyotes (*Canis latrans*) (Levi et al., 2012). Additional work on the importance of predator-prey dynamics would be particularly interesting.

In malaria-endemic regions (e.g., South America and Africa), diagnosis of babesiosis is complicated. In fact, cases in the United States, India, Spain, and Korea were initially diagnosed as malaria and babesiosis was only suspected after treatment failure occurred or after blood smears were carefully screened (Western et al., 1970; Olmeda et al., 1997; Marathe et al., 2005; Kim et al., 2007). However, exposure to *Babesia* in some of these areas appears to be common, based on the limited serologic studies that have been conducted. In both South America and Africa, PCR-based studies with sequence analysis are needed to identify the causative agents of human babesiosis so the surveillance of potential reservoirs and vectors can be conducted. Additionally, the use of PCR assays and/or restriction enzyme assays that distinguish *Babesia* from *Plasmodium* may help to determine the true prevalence of human babesiosis in malaria-endemic areas.

In conclusion, numerous *Babesia* species have been identified as zoonotic, but for many of them, formal descriptions including molecular phylogeny, serological reactivity, and morphologic description are lacking. Importantly, as useful as molecular characterization has become for distinguishing *Babesia*, it is imperative that morphologic and serologic data as well as natural history data (e.g., reservoir host(s) and vector(s)) be investigated because these data are critical to understanding the epidemiology of the pathogens. Surveillance of mammals, birds, and questing ticks are needed, especially in areas where undescribed parasites are emerging, so that genetic sequences can be obtained for future comparison with human cases. Also, continued surveillance of ticks is needed to understand where changes in density and distribution or introductions of new species into naïve areas are occurring. With these data in place, novel human babesiosis agents can be quickly identified and more focused surveillance, public education, and management plans can be implemented.

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