Babesiosis in dogs and cats—Expanding parasitological and clinical spectra

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A R T I C L E   I N F O

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A B S T R A C T

Canine babesiosis caused by different Babesia species is a protozoal tick-borne disease with worldwide distribution and global significance. Historically, Babesia infection in dogs was identified based on the morphologic appearance of the parasite in the erythrocyte. All large forms of Babesia were designated Babesia canis, whereas all small forms of Babesia were considered to be Babesia gibsoni. However, the development of molecular methods has demonstrated that other Babesia species such as Babesia conradae, Babesia microti like plasm, Theileria spp. and a yet unnamed large form Babesia spp. infect dogs and cause distinct diseases. Babesia rossi, B. canis and Babesia vogeli previously considered as subspecies are identical morphologically but differ in the severity of clinical manifestations which they induce, their tick vectors, genetic characteristics, and geographic distributions, and are therefore currently considered separate species. The geographic distribution of the causative agent and thus the occurrence of babesiosis are largely dependent on the habitat of relevant tick vector species, with the exception of B. gibsoni where evidence for dog to dog transmission indicates that infection can be transmitted among fighting dog breeds independently of the limitations of vector tick infestation. Knowledge of the prevalence and clinicopathological aspects of Babesia species infecting dogs around the world is of epidemiologic and medical interest. Babesiosis in domestic cats is less common and has mostly been reported from South Africa where infection is mainly due to Babesia felis, a small Babesia that causes anemia and icterus. In addition, Babesia cati was reported from India and sporadic cases of B. canis infection in domestic cats have been reported in Europe, B. canis presentii in Israel and B. vogeli in Thailand. Babesiosis caused by large Babesia spp. is commonly treated with imidocarb dipropionate with good clinical response while small Babesia spp. are more resistant to anti-babesial therapy. Clinical and parasitological cure are often not achieved in the treatment of small Babesia species infections and clinical relapses are frequent. The spectrum of Babesia pathogens that infect dogs and cats is gradually being elucidated with the aid of molecular techniques and meticulous clinical investigation. Accurate detection and species recognition are important for the selection of the correct therapy and prediction of the course of disease.

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1. Definition and causes

Babesia species are tick-borne apicomplexan parasites of erythrocytes that infect a variety of domestic and wild animals, and humans. At the end of the 19th century Dr. Victor Babes identified micro-organisms present in the...
erythrocytes of cattle in Rumania as the cause of bovine haemoglobinuria or red water fever. He later found similar organisms also in the red blood cells of sheep. These agents were subsequently named Babesia bovis and Babesia ovis, respectively, and the genus was named Babesia in honor of Dr. Babes (Uilenberg, 2006). Although bovine babesiosis is the oldest tick-borne disease reported, the first record of canine Babesia infection in Europe was made in Italy in 1895 not long after the detection of the bovine disease (Roncalli Amici, 2001).

The genera Babesia and Theileria belong to the phylum Apicomplexa, class Piroplasmmata and order Pirophilida. Babesia and Theileria are related structurally, functionally and phylogenetically to Plasmodium species which cause malaria (Kuo et al., 2008; Lau, 2009). The terms sporozoite, merozoite and trophozoite used for the description of life stages in the life cycle of Babesia are also used for Plasmodium spp.

Babesiosis caused by different Babesia species is a disease with a worldwide distribution characterized by erythrocyte destruction causing mild to severe systemic clinical manifestations (Boozer and Macintire, 2003). Historically, Babesia infection in dogs was identified based on the morphologic appearance of the parasite in the erythrocyte. All large forms of Babesia (2.5–5.0 μm) were designated Babesia canis whereas all small forms (1.0–2.5 μm) were considered as Babesia gibsoni (Boozer and Macintire, 2003). However, the development of molecular methods based on the characterization of the nuclear small subunit-ribosomal RNA gene (18S rDNA), the first and second internal transcribed spacers (ITS1, ITS2) loci as well as the intervening 5.8S coding region of the rRNA gene and hsp 70 (Yamasaki et al., 2007) and cytchrome B (Criado et al., 2006) genes have demonstrated that more piroplasmid species infect dogs. These include the small piroplasms Babesia conradae (Kjemtrup and Conrad, 2006; Kjemtrup et al., 2006), Babesia microti-like piroplasm (Zahler et al., 2000) which is also referred to as Theileria annae (Camacho-Garcia, 2006; Zahler et al., 2000) or “Spanish dog isolate” (Yeagley et al., 2009), Theileria spp. (Matjila et al., 2008c) and the yet unnamed large form Babesia spp. (Birkheuer et al., 2004b) in addition to those initially described (Carret et al., 1999; Matjila et al., 2004; Zahler et al., 1998) (see Table 1). Babesia rossi, B. canis and Babesia vogeli previously considered as subspecies of B. canis are identical morphologically but demonstrate tremendous variations in geographic distribution, vector specificity, genetic characteristics, and the clinical signs which they induce in dogs, and are therefore currently considered separate species (Caccio et al., 2002; Carret et al., 1999; Irwin, 2009; Zahler et al., 1998). B. microti-like infections have been reported in foxes in North America (Birkheuer et al., 2010) and in Spain (Gimenez et al., 2009). In addition, molecular detection of B. rossi infection was found in African wild dogs in South Africa (Matjila et al., 2008a). The geographical distribution of the causative agent and thus the occurrence of babesiosis is largely dependent on the habitat of relevant vector tick species. Knowledge of the prevalence and clinicopathological conditions caused by Babesia species infecting dogs and wild canids around the world is of epidemiologic and medical interest.

Babesiosis in domestic cats is a more rare clinical infection in comparison with its canine counterpart. Clinical domestic feline babesiosis has mostly been reported from South Africa where infection is mainly due to Babesia felis, a small Babesia that causes anemia and icterus (Penzhorn et al., 2004; Schoeman et al., 2001). B. felis also infects African wild felids including lions, cheetahs and servals (Bosman et al., 2007). Other reports of domestic feline babesiosis have mostly been sporadic. Babesia cati was reported from a cat in India (Mudaliar et al., 1950) and a few cases of infection in domestic cats by unnamed Babesia parasites were reported in France, Germany, Thailand and Zimbabwe (Bourdeau, 1996; Jittapalapong and Jansawan, 1993; Moik and Gothe, 1997; Stewart et al., 1980). A large form Babesia, B. canis presentii, was described in cats from Israel (Baneth et al., 2004). Interestingly, the presence of Babesia species typical to dogs in domestic cats is detected sporadically by molecular techniques often without compelling evidence of clinical infection. Molecular evidence for infection by B. canis in cats was provided in a study from Spain and Portugal in which a partial DNA sequence from the small subunit RNA gene identified as belonging to B. canis was amplified from three cats and the B. microti-like piroplasmid from two cats (Criado-Fornelio et al., 2003b). In addition, B. vogeli has been identified by blood smear examination and PCR in stray cats from metropolitan Bangkok, Thailand (Simking et al., 2010). Another small piroplasm infecting felines is Cytotauxzon felis which infects the bobcat (Lynx rufus) and domestic cats. It is related to Theileria and Babesia and is endemic in the United States (Holman and Snowden, 2009; Meinkoth and Kocan, 2005). Molecular recognition of Cytotauxzon-like parasites has been reported in domestic cats (Criado-Fornelio et al., 2004) and in Iberian Lynx (Millan et al., 2007, 2009) in Spain and in a domestic cat in France (Criado-Fornelio et al., 2009).

2. Pathophysiology

2.1. Epidemiology, transmission, life cycle

2.1.1. Geographic distribution

The world-wide geographic distribution of canine babesiosis is described in Table 1. B. rossi has to date been restricted to Africa and B. canis has mostly been reported from Europe, whereas B. vogeli and B. gibsoni have wide distributions in both the Old and New world continents. An interesting situation exists in Europe where several Babesia species have been described (Fig. 1). Molecular studies on canine Babesia infection in Europe have demonstrated B. canis infection in Croatia, Poland (Caccio et al., 2002), Hungary (Foldvari et al., 2005), Russia (Rar et al., 2005), Switzerland (Porchet et al., 2007) and Germany (Zahler et al., 1998). Infection with both B. canis and B. vogeli has been reported in Slovenia (Duh et al., 2004), France (Caccio et al., 2002), Spain (Criado-Fornelio et al., 2007), Portugal (Cardoso et al., 2008) and Albania (Hamel et al., 2009). In Italy, B. canis is mainly described in the north and less frequently in central Italy while B. vogeli is predominantly found in central and southern Italy (Solano-Gallego et al., 2008). Additionally, canine B. vogeli infections have been
### Table 1
Distribution, vectors, and cytological characteristics of selected *Babesia* and *Theileria* spp. that infect dogs (modified from Solano-Gallego, 2008).

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic distribution</th>
<th>Proven or putative vector(s)</th>
<th>Size (μm)</th>
<th>Cytological appearance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. rossi</em></td>
<td>South Africa, Nigeria, Sudan</td>
<td><em>Haemophysalis elliptica</em> (formerly <em>H. leachi</em>)</td>
<td>2 × 5</td>
<td>Usually paired</td>
<td>Matjila et al. (2008b), Oyamada et al. (2005), Sasaki et al. (2007)</td>
</tr>
<tr>
<td><em>B. canis</em></td>
<td>Europe</td>
<td><em>Dermacentor spp.</em>, <em>Rhipicephalus sanguineus</em></td>
<td>2 × 5</td>
<td>Usually paired</td>
<td>Bourdoiseau (2006), Cassini et al. (2009), Iori et al. (2010)</td>
</tr>
<tr>
<td><em>B. vogeli</em></td>
<td>Africa, Asia, Europe, North/Central/South America, Australia</td>
<td><em>R. sanguineus</em></td>
<td>2.5 × 4.5</td>
<td>Single or paired</td>
<td>Criado-Fornelio et al. (2007), Jefferies et al. (2003), M’Ghirbi and Bouattour (2008), Oyamada et al. (2005), Passos et al. (2005), Sasaki et al. (2007)</td>
</tr>
<tr>
<td><strong>Unnamed large form Babesia</strong></td>
<td>Eastern USA</td>
<td>Unknown</td>
<td>2x6</td>
<td>Ameboid, paired piriform</td>
<td>Birkenheuer et al. (2004b), Sikorski et al. (2010)</td>
</tr>
<tr>
<td><em>B. gibsoni</em></td>
<td>Southeast Asia, United States, South America, Australia, Europe</td>
<td><em>H. longicornis</em>, <em>H. bispinosa</em>?</td>
<td>1 × 3</td>
<td>Usually singular</td>
<td>Birkenheuer et al. (2005), Hartelt et al. (2007), Jefferies et al. (2007b), Miyama et al. (2005), Trapp et al. (2006)</td>
</tr>
<tr>
<td><em>B. conradae</em></td>
<td>USA (California)</td>
<td><em>R. sanguineus</em>?</td>
<td>0.3-3</td>
<td>Ring, tetrad, ameboid</td>
<td>Kjemtrup and Conrad (2006)</td>
</tr>
<tr>
<td><em>B. microti</em>-like (<em>Theileria annae</em>)</td>
<td>Spain (Galicia, Burgos), Croatia, North Americaa</td>
<td><em>Ixodes hexagonus</em>, <em>I. ricinus</em>, <em>R. sanguineus</em>?</td>
<td>1 × 2.5</td>
<td>Usually singular</td>
<td>Beck et al. (2009), Camacho et al. (2003), Gimenez et al. (2009), Lledó et al. (2010), Iori et al. (2010), Yeagley et al. (2009)</td>
</tr>
<tr>
<td><em>Theileria sp.</em></td>
<td>South Africa</td>
<td>Unknown</td>
<td>Molecular detection only</td>
<td>Not known</td>
<td>Matjila et al. (2008c)</td>
</tr>
</tbody>
</table>

*a* The *B. microti*-like piroplasm has been described in dogs in Galicia, Croatia, and the USA and in foxes in Burgos (Spain) and North America (USA and Canada).

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**Fig. 1.** The distribution of canine *Babesia* species in Europe is shown based mainly on molecular analysis. Note the presence of *B. vogeli* mainly around the Mediterranean basin and infection with *B. canis* mostly in the cooler climate zones of north and central Europe. The references for each country are included in the reference list.
reported in Turkey (Gulanber et al., 2006) and recently, the first case of autochthonous babesiosis caused by *B. canis* in a dog was reported from Norway (Oines et al., 2010). An outbreak of autochthonous canine babesiosis caused by *B. canis* was also reported in the Netherlands (Matjila et al., 2005). *B. microti*-like piroplasm infections were reported in north-western Spain (Camacho-Garcia, 2006; Camacho et al., 2001) and have since been detected also in Croatia (Beck et al., 2009) and the United States (Yeagley et al., 2009). Occasional clinical cases of *B. gibsoni* were reported in Spain (Criado-Fornelio et al., 2003a), Germany (Hartelt et al., 2007) and Italy (Trotta et al., 2009). In addition, recent molecular surveys have reported sporadic *Theileria equi*, *Theileria annulata* and *Babesia caballi* infection detected only by PCR in dogs from Spain (Criado-Fornelio et al., 2007), Croatia (Beck et al., 2009) and France (Fritz, 2010). However, the epidemiologic and clinical significance of these infections in dogs is unknown.

2.1.2. Transmission

The natural transmission of babesiae to vertebrate hosts occurs through the bite of a vector tick. Although information on some of the tick vectors for canine babesial species is available (Table 1), very little is known regarding the tick transmission of feline babesial species. *B. gibsoni* infection has also been demonstrated to be transmitted via blood transfusion (Stegeman et al., 2003), contaminated equipment and transplacentally (Fukumoto et al., 2005a). In addition, several studies have provided evidence that *B. gibsoni* is likely transmitted directly from dog to dog via bite wounds, saliva, or ingested blood (Birkenheuer et al., 2005; Jeffries et al., 2007b; Yeagley et al., 2009).

*B. gibsoni* is endemic in Southeast Asia. Although *B. gibsoni* may infect any dog, a high prevalence of infection is found in fighting dog breeds such as the Pit Bull Terrier and the Tosa (Lee et al., 2009; Miyama et al., 2005). This infection appears to be transmitted mostly by two modes of transmission: tick bites and directly from dog to dog (Irwin, 2009). Tick bites appear to be the most common mode of transmission in Southeast Asia, where infection is commonly observed in both fighting and non-fighting dog breeds (Konishi et al., 2008). In contrast, *B. gibsoni* infection in the USA (Birkenheuer et al., 2003b, 2005; Yeagley et al., 2009) and Australia (Jeffries et al., 2007b) is found mostly in Pitt Bull Terriers. Studies from these countries have indicated that direct dog to dog transmission is highly likely and it might possibly be the main mode of transmission (Birkenheuer et al., 2005; Jeffries et al., 2007b; Yeagley et al., 2009). Dogs with history or evidence of bites are much more likely to be infected with *B. gibsoni* (Birkenheuer et al., 2005; Yeagley et al., 2009). A hundred and fifty Pitt Bull-type dogs confiscated from dog fighting operations in the USA were compared in one study to 218 other dogs selected randomly from dog shelters with no history of dog fighting (Yeagley et al., 2009). PCR for *B. gibsoni* indicated that 34% of the confiscated dogs were positive for *B. gibsoni* while of the control dogs, only 1 dog (0.5%) was positive for *B. gibsoni*. None of the *B. gibsoni* PCR positive dogs was positive for any other tick-borne rickettsial pathogen as might be expected if transmission was mostly by tick bites. Dogs with scars on the head and forelimbs indicative of fighting were five times likely to have *B. gibsoni* infection in this study (Yeagley et al., 2009).

2.1.3. Babesia life cycle

Some species of *Babesia* including *B. canis* have been shown to be infective to animals only 2–3 days after tick attachment (Schein et al., 1979). It is presumed that the change in temperature or the presence of a blood meal in the tick gut acts as an activation stimulus for the maturation of the infective sporozoites. Animals are infected when *Babesia* sporozoites are injected with saliva into the host’s skin during the blood meal. In the host, parasites attach to erythrocyte membranes and invade the cell cytoplasm where they form ring-shaped trophozoites. The parasite replicates by budding within the erythrocyte and forms merozoites observed as pairs of attached pear-shaped parasites in some *Babesia* species. Merozoites may further divide forming 8 or more parasites in the same RBC and eventually destroying the cell and become free in the blood to invade new cells. Ticks feeding on infected blood take up merozoites and sexual parasite development in the tick gut is followed by sporogony in the tick tissues. The parasite reaches the tick salivary glands and oocytes from which transmission occurs (Chauvin et al., 2009). In general, *Babesia* spp. are transmitted trans-stadially from one stage in the tick life cycle to another, and also transovarially through the tick eggs, as shown for some *Babesia* spp. and may therefore be passed through generations of ticks without having to feed on an infected host (Chauvin et al., 2009; Taboada and Lobetti, 2006; Uilenberg, 2006). The life cycle of *Babesia* spp. in the mammalian host takes place exclusively in erythrocytes, whereas *Theileria* spp. have pre-erythrocytic life stage in leukocytes (Chauvin et al., 2009; Uilenberg, 2006).

2.2. Pathogenesis

The pathology of *Babesia* infects in the host varies considerably with the different species and sub-species involved, and also with the host's individual immune status, age, concurrent infections or illness and response to infection (Irwin, 2009). Haemolytic anaemia and systemic inflammatory response syndrome leading to multiple-organ dysfunction syndrome account for most of the clinical signs observed in canine and feline babesiosis (Taboada and Lobetti, 2006). In general, *Babesia* species cause a haemolytic anaemia which is multifactorial and is the predominant clinical manifestation inducing a number of immune responses that may have a devastating influence (Ayoob et al., 2010a). Haemolytic anaemia can occur due to direct red blood cell lysis by replicating intracellular parasites which cause a combination of intravascular and extravascular haemolysis. A number of mechanisms are involved with red blood cell destruction. These include the binding of antibodies to cell surface and complement activation (Adachi et al., 1994, 1995; Carli et al., 2009), production of serum haemolytic factors (Onishi et al., 1990), erythrocyte oxidative damage and increased red blood cell phagocytosis (Murase et al., 1996; Otsuka et al., 2001, 2002), creation of spherocytes, and a decrease in the osmotic fragility of red blood cells (Makinde and Bobade, 2011).
Antibodies against red blood cells have been documented in dogs infected with *B. gibsoni* (Adachi and Makimura, 1992; Adachi et al., 1994) and *B. vogeli* but not in *B. canis* (Carli et al., 2009). Intense haemolysis results in haemoglobinemia, haemoglobinuria, bilirubinemia and bilirubinuria.

Thrombocytopenia alone is observed in many cases of babesiosis and may relate to immune, splenic sequestration or coagulatory consumption of platelets from haemolytic or vascular injury. Immune mediated thrombocytopenia has been demonstrated in experimental canine babesiosis caused by *B. gibsoni* (Wilkerson et al., 2001). However, abnormal coagulation parameters have not been reported frequently in dogs with babesiosis (Taboada and Lobetti, 2006).

Tissue hypoxia is an important contributor to many of the clinical signs caused by the most *Babesia* spp. and studied in-depth in *B. rossi* infection (Leisewitz et al., 2001; Jacobson, 2006). Causes of hypoxia include anemia, hypotensive shock, vascular stasis by sludging of erythrocytes, excessive endogenous production of carbon monoxide, parasitic damage to haemoglobin and decreased ability of haemoglobin to offload oxygen from *Babesia*-infected dogs (Ayoob et al., 2010a; Taboada and Lobetti, 2006). The central nervous system, kidney, and muscle are the organs most affected by the resultant tissue hypoxia (Jacobson, 2006). Hypoxia is thought to be more important than haemoglobinuria in damaging the kidneys of dogs with babesiosis (Ayoob et al., 2010a; Lobetti et al., 1996; Mathe et al., 2007). The main histological changes observed in the kidneys in naturally acquired *B. canis* infections were vascular-hydropic degeneration, necrosis and detachment of renal tubular epithelial cells in the proximal convoluted tubules while no significant histological changes were seen in the glomeruli (Mathe et al., 2007). Tubular haemoglobin casts and haemoglobin droplets in the renal tubular epithelial cells were rarely observed (Mathe et al., 2007). These histological changes were most consistent with hypoxic damage (Mathe et al., 2007).

Tissue hypoxia, hypertensive shock, multiple organ dysfunction and potential mortality have been documented mostly in association with *B. rossi* infection (Jacobson, 2006; Reyers et al., 1998). Infection with this species may present acutely or even as a peracute and fatal syndrome with massive haemolysis, renal failure and acid–base abnormalities (Leisewitz et al., 2001). Free oxygen radical release and mechanisms associated with harmful cytokine effects have been associated with endothelial damage and increased vascular permeability in canine babesiosis. These may result in non-cardiogenic pulmonary edema (Jacobson, 2006). Cerebral babesiosis has also been documented with *B. rossi* (Jacobson, 2006). The clinical manifestations of infection with other large *Babesia* spp. are usually less severe and typically vary from a mild to moderate disease with *B. vogeli* to a moderate disease with *B. canis* (Taboada and Lobetti, 2006).

The spleen has an important function in controlling babesiosis (Homer et al., 2000). Splenectomized dogs that are experimentally infected rapidly develop parasitaemia and clinical disease (Vercammen et al., 1995) and may reach high parasitaemia levels. Splenectomy is an important risk factor for the development of natural and potentially fatal babesiosis in humans (Boustanian and Gelfand, 1996; Gorenflo et al., 1998; Rosner et al., 1984) and has also been documented to be associated with clinical natural canine babesiosis (Camacho et al., 2002; Sikorski et al., 2010; Solano-Gallego et al., 2008).

3. Clinical signs and clinicopathological abnormalities

3.1. Canine babesiosis

3.1.1. *B. rossi*

*B. rossi* is considered to cause the most severe disease manifestations, among the large babesial species that infect dogs and the disease is most prevalent in summer (Jacobson, 2006; Reyers et al., 1998). Dogs infected with *B. rossi* may present clinical manifestations which have been categorized as (1) uncomplicated or with a relatively uncompromised blood circulatory system (with a good prognosis) or (2) complicated or with a clinically compromised circulation (with a poor prognosis) (Bohm et al., 2006; Jacobson, 2006; Reyers et al., 1998). The disease is categorized as having a more favorable prognosis if only mild or moderate anemia, with no clinical evidence of organ dysfunction or failure is present. Dogs in this category are treated with antibabesial drugs and, if needed also with blood transfusion, and have been reported to usually recover successfully and have a high survival rate. In contrast, cases of the disease which circulatory compromise are those where clinical presentation is complicated by evidence of organ failure characterized by severe anemia and haemococoncentration or specific organ dysfunction (Jacobson, 2006). These dogs usually require intensive treatment at a veterinary medical emergency care facility. The clinical manifestations and clinicopathological abnormalities of both disease categories are summarized in Table 2. The mortality rate in dogs with complicated babesiosis is around 15%, irrespective of the nature of the treatment administered (Jacobson, 2006). Many factors including high parasitaemia and state of collapse at presentation (Bohm et al., 2006), hypoglycaemia (Keller et al., 2004) and high serum lactate that fails to decrease after 24 h (Nel et al., 2004), high cortisol and ACTH concentrations and low thyroxine and free thyroxine concentrations (Schoeman et al., 2007), as well as cerebral, lung and renal involvement (Welzl et al., 2001) have been shown to be associated with mortality in the virulent form of canine babesiosis found in South Africa. Interestingly, a polymorphic phosphoprotein localized on the cytoplasmic surface of *B. rossi*-infected red blood cells has recently been characterized and named *B. rossi* erythrocyte membrane antigen 1 (BrEMA1). This protein is suspected as a virulence factor in *B. rossi* canine babesiosis (Matjila et al., 2009). Curiously, less pathogenic species of *Babesia* infecting dogs such as *B. canis* and *B. vogeli* do not have the BrEMA1 gene. A preliminary study suggests that there are also clinically important differences between various *B. rossi* genotypes (Matjila et al., 2009).
<table>
<thead>
<tr>
<th>Species (host)</th>
<th>Clinical data</th>
<th>Clinical manifestations</th>
<th>Clinicopathological abnormalities</th>
<th>Prognosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. canis</em></td>
<td>Young adult, outdoor, hunting/shepherd dogs</td>
<td>Fever, lethargy, anorexia, jaundice</td>
<td>Thrombocytopenia, mild to moderate normocytic non regenerative anemia, infrequent regenerative anemia, neutropenia, pigmenturia, bilirubinemia, bilirubinuria (due to haemolysis)</td>
<td>Good</td>
<td>Bourdoiseau (2006), Carli et al. (2009), Solano-Gallego et al. (2008)</td>
</tr>
<tr>
<td><em>B. vogeli</em></td>
<td>Pups or adult dogs with concomitant diseases</td>
<td>Fever, lethargy, anorexia, jaundice</td>
<td>Haemolytic regenerative immune mediated anemia, non regenerative anemia, leukocytosis, leucopenia, thrombocytopenia</td>
<td>Good</td>
<td>Carli et al. (2009), Solano-Gallego et al. (2008)</td>
</tr>
<tr>
<td><em>B. rossi</em></td>
<td>Young or adult dogs</td>
<td>Uncomplicated fever, lethargy, anorexia, pale mucous membranes, splenomegaly</td>
<td>Mild to moderate anemia, thrombocytopenia, leukocytosis, pigmenturia, bilirubinemia, bilirubinuria</td>
<td>Good</td>
<td>Jacobson (2006), Keller et al. (2004), Leisewitz et al. (2001)</td>
</tr>
<tr>
<td><em>B. conradae</em></td>
<td>Described in dogs from Southern California</td>
<td>Lethargy, pale mucous membranes, vomiting, lymphadenomegaly</td>
<td>Haemolytic immune mediated regenerative anemia, thrombocytopenia</td>
<td>Guarded to poor</td>
<td>Kjemtrup and Conrad (2006)</td>
</tr>
<tr>
<td>Unnamed large form <em>Babesia</em></td>
<td>Splenectomized or immuno-compromised dogs (Pit-bull Terriers, Tosa)</td>
<td>Fever, lethargy, anorexia</td>
<td>Mild non regenerative anemia and thrombocytopenia</td>
<td>Good</td>
<td>Birkenheuer et al. (2004b), Sikorski et al. (2010), Birkenheuer et al. (1999), Lee et al. (2009), Macintire et al. (2002), Meinkoth et al. (2002)</td>
</tr>
<tr>
<td><em>B. gibsoni</em></td>
<td>Common in fighting dogs</td>
<td>Fever, lethargy, pale mucous membranes, jaundice, lymphadenomegaly, splenomegaly, weight loss</td>
<td>Haemolytic regenerative anemia, sometimes immune mediated anemia, thrombocytopenia, pigmenturia, bilirubinemia, bilirubinuria</td>
<td>Guarded</td>
<td>Birkenheuer et al. (1999), Lee et al. (2009), Macintire et al. (2002), Meinkoth et al. (2002)</td>
</tr>
<tr>
<td><em>B. microti-like</em></td>
<td>Young adult dogs, outdoor dogs (guardian or hunting dogs)</td>
<td>Weakness, fever, lethargy, pigmenturia, tachycardia, tachypnea</td>
<td>Moderate to severe regenerative anemia, thrombocytopenia, azotemia, proteinuria, urinary casts</td>
<td>Guarded to poor</td>
<td>Camacho-Garcia (2006), Camacho et al. (2004), Guitian et al. (2003)</td>
</tr>
</tbody>
</table>
3.1.2. *B. canis*

*B. canis* causes a mild to severe disease in which parasitaemia is often low and does not necessarily correlate with the severity of clinical illness (Uilenberg et al., 1989). The main acute clinical signs are fever, anaemia, lethargy and dehydration. The majority of dogs infected with *B. canis* present with mild to severe thrombocytopenia, hyperfibrinogenemia, mild to moderate normocytic-normochromic non-regenerative anaemia, haemolysis and neutropenia (Table 2). Haemoglobinuria has been described in naturally infected dogs (Bourdoiseau, 2006; Solano-Gallego et al., 2008). Experimental infection with *B. canis* resulted in transient low parasitaemia (1–2%), a decrease in PCV, thrombocytopenia, an increase in the activated partial thromboplastin time (APTT), and hypotension (Schetters et al., 1997, 2009a). In addition, babesiosis caused by *B. canis* predominantly occurs during autumn or spring due to the favorable conditions for tick vector activity (Bourdoiseau, 2006; Cardoso et al., 2010; Mathe et al., 2006; Solano-Gallego et al., 2008).

3.1.3. *B. vogeli*

*B. vogeli* usually causes a subclinical to mild or moderate clinical disease (Carret et al., 1999; Uilenberg et al., 1989) which often accompanies other diseases or affects splenectomized dogs. Severe to fatal haemolytic anaemia is possible in young dogs and pups (Solano-Gallego et al., 2008). Adult Greyhounds in the U.S. sero-reactive for *B. vogeli* are commonly clinically healthy (Taboada et al., 1992; Taboada and Lobetti, 2006). Haemolytic regenerative immune mediated anaemia (Carlé et al., 2009) is a common finding in *B. vogeli* infection but a homogenous clinicopathological pattern is frequently not found in contrast to the more uniform inflammatory patterns described in *B. canis* infections (Solano-Gallego et al., 2008). The main clinical signs, laboratory abnormalities found in *B. vogeli* infection, and the treatment reported to be effective are described in Tables 2 and 3.

3.1.4. The unnamed large form Babesia

The currently unnamed large form Babesia was described for the first time in North Carolina in a dog under chemotherapy for lymphoma (Birkenheuer et al., 2004b). Recently, seven dogs infected with this pathogen have been described in the eastern USA. All the dogs presented with immunocompromised conditions such as splenectomy or chemotherapy due to neoplasia (Sikorski et al., 2010). Analyses of the 18S rRNA gene of the unnamed large form Babesia have revealed a unique sequence that shared a 93.9% identity with *B. bigemina* (Birkenheuer et al., 2004b). The main clinical signs and laboratory abnormalities found in this infection and the treatment instituted are described in Tables 2 and 3. All dogs were initially diagnosed as having babesiosis by microscopic examination of thin blood smear and most of them responded well to imidocarb dipropionate treatment (Sikorski et al., 2010).

3.1.5. *B. gibsoni*

Chronic infection is common and manifested as a subclinical infection or associated with weight loss and weakness. Subclinical infection is common in Pit Bull Terriers in the USA and found in the majority of dogs from this breed examined in one study (Birkenheuer et al., 2005).

The main clinical signs, laboratory abnormalities, and treatment protocols described for *B. gibsoni* are outlined in Tables 2 and 3.

3.1.6. *B. conradae*

*B. conradae* infection has been described from California in the western USA and was previously considered as caused by *B. gibsoni* until characterized genetically. *B. conradae* seems to be more virulent than *B. gibsoni* infection resulting in higher parasitaemia, more pronounced anaemia and higher rate of mortality (Conrad et al., 1991; Kjemtrup and Conrad, 2006). The clinical findings are similar to those reported in *B. gibsoni* infections (Kjemtrup and Conrad, 2006) and are described in Table 2.

3.1.7. *B. microti-like piroplasm (T. annae)*

The most common clinical findings reported in dogs infected with this relatively recently described small piroplasm from the north west of Spain are listed in Table 2 (Camacho-Garcia, 2006; Camacho et al., 2004). The majority of cases are observed in autumn and winter (Camacho-Garcia, 2006). Azotemia appears to be a common complication of this infection. In a study describing 58 *B. microti*-like infected dogs, 36% of the dogs were azotemic at the time of diagnosis (Camacho et al., 2004, 2005). Additional studies are needed to separate pre-renal from renal azotemia in *B. microti*-like infected dogs.


A *Theileria* species was detected by PCR in blood samples collected from dogs in South Africa (Matjila et al., 2008c). Phylogenetic analysis of the 18S rRNA full-length gene sequences of this parasite revealed a close relationship with sequences of *Theileria* species found in the sable antelope. The clinical significance of this infection in dogs is currently poorly understood and the clinicopathological manifestations found in infected dogs included haemolytic anaemia, splenomegaly, and thrombocytopenia (Matjila et al., 2008c).

3.2. Feline babesiosis

*Babesia* infection in cats is associated with anaemia, lethargy, anemia and icterus. Information on the clinical manifestations of domestic feline babesiosis is limited mostly to publications on *B. felis* infection in South Africa (Ayoob et al., 2010b; Penzhorn et al., 2004; Schoeman et al., 2001). In a study on *B. felis* that included 56 cats (Schoeman et al., 2001), 80% were less than 3 years old and there was no specific breed or gender predilection. Most cats were anorectic and lethargic. Macrocytic hypochromic regenerative anaemia was present in the majority of infected cats. Hyperbilirubinemia was present in 86% of the cats and alanine aminotransferase activity was elevated in 89%. Thirty two percent of the cats were concurrently infected with feline leukemia virus (FeLV) and 14% with feline immunodeficiency virus (FIV). *Babesia canis presentii* infection in a cat from Israel co-infected with FIV and *Candidatus Mycoplasma haemominutum* was accompanied by fever,
Table 3

<table>
<thead>
<tr>
<th>Babesia species (host)</th>
<th>Drug</th>
<th>Dose and duration</th>
<th>Response to treatment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. canis; B. vogeli and B. rossi (Dog)</td>
<td>Imidocarb dipropionate</td>
<td>5–6.6 mg/kg IM once; may repeat in 14 days</td>
<td>Good</td>
<td>Vial and Gorenflo (2006)</td>
</tr>
<tr>
<td>Unnamed large form Babesia (Dog)</td>
<td>Diminazene aceturate</td>
<td>A single dose of 3–5 mg/kg</td>
<td>Good</td>
<td>Jacobson et al. (1996)</td>
</tr>
<tr>
<td>B. gibsoni (Dog)</td>
<td>Imidocarb dipropionate</td>
<td>5–6.6 mg/kg IM once; may repeat in 14 days</td>
<td>Improvement of anemia and clinical signs without elimination of parasite and with occasional to frequent clinical relapses</td>
<td>Birkenheuer et al. (2004a), Jefferies et al. (2007c), Matsu et al. (2004), Sakuma et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Azithromycin + atovaquone</td>
<td>10 mg/kg PO SID + 13.3 mg/kg PO TID for 10 days</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>12–25 mg/kg PO BID for 7–10 days</td>
<td>Moderate to poor with frequent relapses</td>
<td>Kulansari et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>B. conradae (Dog)</td>
<td>Imidocarb dipropionate</td>
<td>5–6.6 mg/kg IM once; may repeat in 14 days</td>
<td>Poor</td>
<td>Kjertrup and Conrad (2006)</td>
</tr>
<tr>
<td>B. microti-like (Dog)</td>
<td>Diminazene aceturate</td>
<td>A single dose of 3–5 mg/kg</td>
<td>Poor</td>
<td>Camaaro-Garcia (2006)</td>
</tr>
<tr>
<td>B. felis (Cat)</td>
<td>Imidocarb dipropionate</td>
<td>0.5 mg/kg PO SID for 3 days. Note: &gt;1 mg/kg is lethal in cats.</td>
<td>Good but with occasional to frequent relapses</td>
<td>Ayoob et al. (2010b), Penzhorn et al. (2004)</td>
</tr>
<tr>
<td>B. canis presentii (Cat)</td>
<td>Imidocarb dipropionate</td>
<td>2.5–3 mg/kg IM</td>
<td>Good</td>
<td>Baneth et al. (2004)</td>
</tr>
</tbody>
</table>

Icterus, moderate anemia and thrombocytopenia which resolved following anti-babesial therapy (Baneth et al., 2004). The only systemic abnormality recorded in a cat with B. catti infection in India was fever (Mudaliar et al., 1950).

4. Diagnosis

4.1. Detection of babesiae in stained blood smears or by flow cytometry

Detection of Babesia in stained blood smears has been the standard diagnostic technique for many years. This method is reliable when a moderate to high parasitaemia is present. However, a direct correlation between the level of Babesia parasitaemia and the magnitude of clinical signs is not always found. The diagnosis of chronically infected and carrier dogs remains a diagnostic challenge due to low and often intermittent parasitaemia that is frequently difficult to observe only by microscopic evaluation. Therefore, the use of molecular diagnostic assays is strongly recommended in those cases. Smears made from capillary blood (from ear tip or toe nail) may be beneficial in exhibiting large form Babesia parasites versus blood from a central vein (Bohm et al., 2006). Furthermore, evaluation of smears prepared from a concentrated and stained buffy coat may facilitate diagnosis, as some Babesia organisms preferentially parasitize reticulocytes over mature erythrocytes (Irwin and Hutchinson, 1991). A fresh smear is recommended for the accurate diagnosis of infection. The cytological appearance of different Babesia species is described in Table 1 and it is relatively easy to separate large from small piroplasms under the microscope. However, distinguishing between small or between large canine and feline Babesia species based solely on morphology is not possible and molecular analysis is required for speciation. Automated flow cytometric techniques have been employed to detect Babesia in reticulocytes and mature canine erythrocytes with similar sensitivities to microscopic evaluation (Fukata et al., 1996; Uemura et al., 1990).

4.2. PCR assays

The polymerase chain reaction (PCR) is a sensitive and specific diagnostic technique which is frequently employed for the diagnosis of babesiosis. It is particularly useful for detection of infection in dogs with a low parasitaemia levels and for speciation of parasites. A large number of PCR assays and protocols using a variety of gene targets have been described. A semi-nested PCR able to detect and discriminate DNA from B. canis, B. rossi, B. vogeli and B. gibsoni has been described (Birkenheuer et al., 2003a). In addition, a reverse line blotting (RLB) technique in which PCR products are hybridized to a membrane containing specific probes for the several babesial species and possibly also for other pathogens has been developed for simultaneous detection and speciation of piroplasms and co-infections. The RLB confirmed the presence of B. vogeli in addition to B. rossi in dogs from South Africa (Matija et al., 2004). PCR-restriction fragment length polymorphism is also used to separate between canine Babesia species (Carret et al., 1999; Jefferies et al., 2007a; Solano-Gallego & Baneth, 2008). Recently, high-resolution melting curve quantitative fluorescence resonance energy transfer-PCR has been developed to discriminate between species based on melting curves analysis (Wang et al., 2010).

4.3. Serologic testing

Serology can indicate a past or present persistent infection. The indirect fluorescent antibody test (IFAT) is the most commonly used test for canine babesiosis (Taboada et al., 1992; Vercammen et al., 1995); however, cross reactivity between different Babesia species and other protozoan parasites occurs (Vercammen et al., 1995; Yamane...
Enzyme-linked immunosorbent assays (ELISA) have been used mostly for research and epidemiologic surveys (Schetters et al., 1996). The use of recombinant proteins such as the thrombospondin-related adhesive protein (TRAP) of *B. gibsoni* has been employed as an alternative for whole parasite antigen with good sensitivities and specificities (Goo et al., 2008). False-negative results are possible in peracute or acute infection. In these cases, the use of convalescent antibody titers is strongly recommended to confirm acute infection.

### 4.4. Treatment

The different drugs, doses, treatment duration and the reported response to treatment in different species of *Babesia* infecting dogs and cats are listed in Table 3. Large forms of *Babesia* are commonly treated with imidocarb dipropionate with good clinical response while small forms of *Babesia* appear to be more difficult to treat and resistant to the conventional drugs that are effective against the large babesial spp. Diminazene aceturate used for the treatment of both large and small babesial spp. infections has a relatively small dose safety margin with a large inter-individual pharmacokinetic variation, and if selected for treatment should be used with caution (Miller et al., 2005). Although several treatment protocols are employed for small forms of *Babesia* (Table 3), clinical and parasitological cure are commonly not achieved in small babesial spp. infections and clinical relapses may occur frequently. *Babesia gibsoni* infection is frequently resistant to treatments with imidocarb dipropionate and diminazene aceturate, the main drugs used for the treatment of large *Babesia* spp., and an alternative therapy with the combination of the anti-malarial atovaquone and the macrolide azithromycin has been recommended for this infection (Birkenhauer et al., 2004a). New drugs such as artesunate (Goo et al., 2010) and epoxomicin (Aboulaila et al., 2010) are being investigated in the search for more effective treatment against small *Babesia* infections. Medical management of infection may require supportive treatments including the administration of intravenous fluids, blood transfusions and the use of anti-inflammatory drugs (Ayoob et al., 2010a,b).

### 4.5. Prevention

Prevention of babesiosis relies mostly on topical and environmental acaricidal treatments aimed at reducing the exposure to vector ticks and pathogen transmission to the dog or cat. Collars, spot on formulations and sprays are the most popular and effective means of controlling tick infestations on individual animals and a variety of products which include permethrin, amitraz, fipronil, imidacloprid and other chemicals for protection of individual animals are available from commercial companies (Berrada and Telford, 2009; Brianti et al., 2010; Last et al., 2007; Otranto et al., 2010). These topical ectoparasiticides either repel ticks and prevent attachment or kill ticks within 24–48 h after application. Decrease of tick burdens in the environment can be achieved using conventional and slow release acaricidal formulations applied by spray or powder. Biologic control by means of organisms pathogenic specifically to ticks may be used in the future as environmental control measures (Fernandes and Bittencourt, 2008). Periodic treatments with imidocarb dipropionate or with doxycycline have shown variable outcomes and are not recommended currently for the routine prophylaxis of canine babesiosis (Uilenberg et al., 1981; Vercaemmen et al., 1996a,b). As *Babesia* species are transmitted by blood product transfusions, it is highly recommended to screen canine blood donors for *Babesia* infection on a regular basis (Wardrop et al., 2005). Non-vectorial transmission of Babesia by blood transfusions and by dog to dog fighting is preventable and should be of special concern as it can be responsible for the incursion of babesiosis into previously non-endemic areas.

Vaccines against *B. canis* are commercially available in some countries in Europe. One vaccine contains culture derived soluble parasite antigens from a homologous *B. canis* stock and a second bivalent vaccine contains culture derived soluble antigens of heterologous origins with a European *B. canis* stock and a South African *B. rossi* addition (Moreau et al., 1989; Schetters, 2005; Schetters et al., 2009b). Both vaccines induce partial protection against disease caused by *B. canis* manifested by decreased severity of clinical signs, parasitaemia or duration of clinical disease induced by infection challenge. While the former vaccine conferred protection only against strains of *B. canis*, the latter was also partially protective against *B. rossi*. Studies to evaluate vaccination against *B. gibsoni* have been reported using several types of vaccination techniques including recombinant antigen and DNA vaccines (Fukumoto et al., 2005b, 2007, 2009).

### 4.6. Human babesiosis and public health considerations

Human babesiosis caused by several *Babesia* species is an important emerging tick-borne zoonotic disease (Gray et al., 2010). A fatal *Babesia divergens* infection reported in 1956 was the first confirmed case of human babesiosis (Skrabalo and Deanovic, 1957). Babesiosis has since then regarded as an important and potentially life threatening zoonotic infection of humans (Homer et al., 2000). *B. divergens*, a parasite of cattle, has been implicated as the most common agent of human babesiosis in Europe, causing severe disease in splenectomized individuals (Hunfeld et al., 2008). Although several *Babesia* species have been involved in human infections worldwide (Gray et al., 2010), the major public health burden on man lies in North America and is due to *B. microti*, especially in the eastern parts of the United States of America (Homer et al., 2000). *B. microti*, a babesial parasite of rodents, has been the cause of more than 300 cases of human babesiosis since 1969, causing mild to severe disease, also in non-splenectomized patients (Homer et al., 2000; Hunfeld et al., 2008). Recently, the first confirmed autochthonous case of human *B. microti* infection has been reported in Europe (Hildebrandt et al., 2007) and several studies have demonstrated the presence of *B. microti* isolates in *Ixodes ricinus* ticks in northern Europe (Hildebrandt et al., 2010; Lempereur et al., 2011; Wielinga et al., 2008). Moreover, a recent study has indicated the presence of zoonotic *B. microti* in rodents in Croatia (Beck et al., 1993).
et al., 2010). Therefore, it is likely that B. microti infection in humans occurs more often in Europe than previously recognized as suggested by serologic evidence (Hunfeld et al., 2002).

In addition, new Babesia species have been recognised as causing human babesiosis (Gray et al., 2010). These include Babesia venatorum (Babesia EU1), a parasite of deer (Duh et al., 2005) transmitted by I. ricinus ticks (Becker et al., 2009), reported in humans from Italy, Germany and Austria (Haselbarth et al., 2007; Herwaldt et al., 2003) and Babesia duncanii reported in humans from California (Conrad et al., 2006). The reasons for the increased incidence of human babesiosis are complex; most likely involving ecological changes, increased awareness of the disease, an increased number of susceptible individuals, such as those infected with HIV or immunocompromised patients (Hommer et al., 2000; Hunfeld et al., 2008) and transmission of Babesia parasites by blood transfusion (Dodd, 2010; Vannier and Krause, 2009).

So far, human infections with Babesia species that infect dogs and cats have not been reported. However, dogs and cats are close companions of people and serve as a source of infected ticks for humans (Lemperre et al., 2011).

5. Conclusions

The spectrum of Babesia pathogens that infect dogs and cats is gradually being elucidated with the aid of new molecular techniques and meticulous clinical investigation. Species of Babesia that cannot be distinguished morphologically cause diverse diseases and are transmitted by different vector ticks. Non-vector transmission by blood transfusion and directly from dog to dog is of special concern and could be responsible for the spread of infection to areas that were previously non-endemic. Accurate detection and species recognition are important for the selection of the correct therapy, predicting the course of disease and for following the epidemiologic trends related to infection by different species globally.

Conflict of interest

The authors declare that there is no conflict of interest.

References


