Veterinary Parasitology 254 (2018) 58–63

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Antiprotozoal treatment of canine babesiosis

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ARTICLE INFO

Keywords:
Imidocarb dipropionate
Diminazene aceturate
Atovaquone
Buparvaquone
Azithromycin
Apicoplast

ABSTRACT

Canine babesiosis is a tick-borne disease caused by several *Babesia* spp. which have different susceptibility to anti-protozoal drugs. A few drugs and drug combinations are used in the treatment of canine babesiosis often without complete parasite elimination leaving treated dogs as carriers which could relapse with clinical disease and also transmit infection further. Although the large form canine babesial species *Babesia canis*, *Babesia vogeli* and *Babesia rossi* are sensitive to the aromatic diamidines imidocarb dipropionate and diminazene aceturate, small form species such as *Babesia gibsoni*, *Babesia conradae* and *Babesia vulpes* (*Theileria annae*) are relatively resistant to these drugs and are treated with the combination of the hydroxynaphthoquinone atovaquone and the antibiotic azithromycin. Azithromycin and other antibiotics that have anti-protozoal properties target the apicoplast, a relict plastid found in protozoa, and exert a delayed death effect. The triple combination of clindamycin, diminazene aceturate and imidocarb dipropionate is also effective against *B. gibsoni* and used to treat atovaquone-resistant strains of this species. Novel drugs and the synergistic effects of drug combinations against *Babesia* infection should be explored further to find new treatments for canine babesiosis.

1. Introduction

Babesia spp. are tick-borne protozoan parasites that belong to the phylum Apicomplexa, class Piroplasmea, order Piroplasmida and family Babesiidae, and infect erythrocytes of domestic and wild animals, and humans. The distribution of canine babesiosis is worldwide and several different species of *Babesia* have been described in dogs (Irwin, 2009; Solano-Gallego and Baneth, 2011). *Babesia* infection causes disease with clinical manifestations that may vary with the different species and strains involved and their specific virulence, and also with factors that determine the host's response to infection such as age, individual immune status, and the presence of concurrent infections or other diseases (Birkenheuer et al., 1999; Jacobson, 2006; Irwin, 2009). Hemolytic anemia with erythrocyte destruction and a systemic inflammatory response, which may lead to organ dysfunction, account for most of the clinical signs observed in canine babesiosis. The disease onset is often acute with affected dogs suffering from fever and lethargy and thereafter may display clinical manifestations of anemia, liver, pulmonary, kidney or cerebral dysfunction, and hemostatic abnormalities including coagulation and electolyte imbalances. Sub-clinical and sub-acute infections have also been described (Lobetti et al., 1996; Leisewitz et al., 2001; Jacobson, 2006; Irwin, 2009; Eichenberger et al., 2016).

Babesia infection in dogs was identified in the past mostly by the morphologic appearance of the parasite in erythrocytes and all large forms were designated *Babesia canis*, whereas small forms of *Babesia* were considered to be *Babesia gibsoni*. Different levels of virulence, dissimilar antigenic properties and specific tick vector transmission competence have led to the notion that there is a larger variety of *Babesia* spp. that infect dogs (Schetters et al., 1997; Zahler et al., 1998; Irwin 2009; Solano-Gallego and Baneth, 2011). This was proven by molecular methods which demonstrated that several genetically distinct *Babesia* spp. cause disease in dogs (Zahler et al., 1998; Carret et al., 1999). These currently recognized species include *B. canis*, *Babesia vogeli*, *Babesia rossi*, *B. gibsoni*, *Babesia conradae* and *Babesia vulpes* (also termed *Theileria annae* and *Babesia microti*-like) (Table 1). A yet unnamed large *Babesia* sp. has also been reported to cause disease in immunosuppressed dogs in the eastern USA (Sikorski et al., 2010).

Several drugs and drug combinations have been reported to be effective against canine babesiosis (Vial and Gorenflot, 2006; Irwin, 2009, Solano-Gallego and Baneth, 2011; Beugnet and Moreau, 2015). The mechanisms of action of these drugs against *Babesia* are largely unknown or not studied in detail and the explanation for the added value of some drug combinations, albeit evident in clinical trials, is not well understood (Vial and Gorenflot, 2006; Birkenheuer et al., 2004a; Lin et al., 2012; Checa et al., 2017). Furthermore, the *Babesia* spp. that infect dogs have dissimilar drug susceptibilities and respond differently to drugs (Irwin, 2009, Solano-Gallego and Baneth, 2011). It is therefore important to treat babesial infections with the most effective anti-protozoal drugs or their combinations and to recognize the treatment's

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https://doi.org/10.1016/j.vetpar.2018.03.001
Received 27 December 2017; Accepted 2 March 2018
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Table 1
The species of *Babesia* that cause canine babesiosis, their geographic distribution, tick vectors, size of merozoites stages and main drugs used for their treatment.

Species	Geographical distribution	Potential or confirmed vectors	Size of merozoite stages in µm	Main drug or drug combination
Babesia canis	Europe	*Dermacentor reticulatus*	2 × 5 (large forms)	Imidocarb dipropionate
Babesia rossi	Southern Africa, Nigeria, Sudan	*Haemaphysalis elliptica, Haemaphysalis leachi*	2 × 5 (large forms)	Diminazene aceturate; imidocarb dipropionate
Babesia vogeli	Africa, Asia, southern Europe, North, Central and South America, Australia	*Rhipicephais sanguineus* sensu lato	2.5 × 4.5 (large forms)	Imidocarb dipropionate
Large unnamed *Babesia*	Eastern United States	Unknown	2 × 6 (large forms)	Imidocarb dipropionate
Babesia gibsoni	Southeast Asia, United States, Australia, Europe	*Haemaphysalis longicornis, Haemaphysalis bispinosa?, R.. sanguineus* s.l.?[a]	1 × 3 (small forms)	Atovaquone and azithromycin; clindamycin and diminazene aceturate and imidocarb dipropionate for atovaquone-resistant strains
Babesia conradae	United States (California)	*R. sanguineus* s.l.?	0.3–3 (small forms)	Atovaquone and azithromycin
Babesia vulpes (*Babesia microti*-like; *Theileria annae*)	Europe, North America	*D. reticulatus?, Ixodes hexagonus?, Ixodes ricinus?, Ixodes canisuga?, R. sanguineus* s.l.?	1 × 2.5 (small forms)	Atovaquone and azithromycin; bupravaquone and azithromycin

[a] ? refers to a suspected vector.

limitations. The purpose of this review is to describe the main drugs currently available and used for the treatment of canine babesiosis. The drugs and drug combinations reviewed here are frequently not registered or approved for the specific indication mentioned by national agencies and their use may therefore extra label (Table 2).

2. Main drugs and drug combinations used in the antiprozoal treatment of babesiosis

2.1. Imidocarb dipropionate

Imidocarb dipropionate is an aromatic diamidine. Several mechanisms of action have been proposed for imidocarb dipropinate including: blockage of the entry of inositol into erythrocytes containing *Babesia*, resulting in starvation of the parasite (McHardy et al., 1986); interference with production or use of polyamines by parasites as found for *Trypanosoma brucei* (Bacchi et al., 1981), or combination with DNA in susceptible babesial species, causing nucleic acid damage and inhibition of cellular repair and replication (Plumb, 2015). Imidocarb dipropionate is excreted via the kidney and liver and eliminated in the urine and feces.

Imidocarb dipropionate is approved by the Food and Drug Administration (FDA) in the United States of America (USA) for treatment of *B. canis* infections in dogs, but it is also effective in the treatment of other large *Babesia* spp. infecting dogs, more than for the small form *Babesia* spp. (Conrad et al., 1991; Birkenheuer et al., 2004a; Checa et al., 2017). The FDA-approved labeled dose of imidocarb dipropionate (Imizol®, Carbesia®) is 6.6 mg/kg intramuscularly (IM) or subcutaneously (SC) with a repeated dose in 2 weeks. Imidocarb dipropionate should not be administered intravenously (IV) in dogs. The adverse effects of imidocarb dipropionate in dogs include pain during injection and cholinergic effects such as salivation, drooling, nasal drip or vomiting. These cholinergic effects can be mitigated by premedicating with atropine at 0.05 mg/kg. Other less frequent effects are panting, restlessness, diarrhea, renal tubular or hepatic necrosis, and injection site inflammation and more rarely ulceration, which usually heals within days to weeks.

2.2. Diminazene aceturate

Diminazene aceturate is an aromatic diamidine, like imidocarb dipropionate. Its mechanism of action against *Babesia* is not well understood and it is suggested that it disrupts the parasite's DNA synthesis and aerobic glycolysis (Plumb, 2015) as reported for *Trypanosoma* and *Leishmania* spp., where diminazene aceturare was found to inhibit DNA replication and mitochondrial respiratory activity (Leon et al., 1977; Bitonti et al., 1986). Diminazene aceturate (Azidin®, Berenil®, Ganasag®, Pirocide®) in not FDA-approved for treatment of canine babesiosis in the USA; however, it is widely used in some other countries including South Africa, where it is the main drug used against canine babesiosis caused by *B. rossi* (Miller et al., 2005). The dosage for canine babesiosis is 3.5 mg/kg IM once (Plumb, 2015). The efficacy of treatment is variable and the drug tends to be more effective against large-form *Babesia* spp.; however, it has toxic adverse effects with severe neurological signs which may be unpredictable even at therapeutic doses, and its safety index is considered low with doses above 10 mg/kg IM or repeated lower doses within a short duration causing a cumulative effect and severe to fatal toxicity (Plumb, 2015). Diminazene is excreted via the kidney and liver and eliminated in the urine and feces. Adverse effects of diminazene aceturate in dogs administered at therapeutic doses include gastrointestinal disturbances such as vomiting and diarrhea, pain and inflammation in the site of injection, transient drop in blood pressure and more rarely neurological signs including ataxia, seizures, and death (Plumb, 2015).

Table 2
Major drugs and drug combinations used for treatment of canine babesiosis.

Drug or combination	Dosage and route	References
Imidocarb dipropionate	6.6 mg/kg IM or SC; repeat dose in 2 weeks.	(Plumb, 2015)
Diminazene aceturate	3.5 mg/kg IM once	(Plumb, 2015)
Atovaquone + azithromycin	Atovaquone 13.3 mg/kg PO q8h and azithromycin 10 mg/kg PO once daily, both drugs for 10 days	(Plumb, 2015)
Buapravaquone + azithromycin	Buparvaquone 5 mg/kg IM twice 48 h apart and azithromycin 10 mg/kg PO once daily for 10 days	(Checa et al., 2017)
Clindamycin + diminazene aceturate + imidocarb dipropionate (for atovaquone-resistant *Babesia gibsoni*)	Clindamycin 30 mg/kg PO q12h; diminazene aceturate 3.5 mg/kg IM once on the day of treatment start; imidocarb diproprionate 6 mg/kg SC once on the day after diminazene is administered.	(Lin et al., 2012)

IM: intramuscular; SC: subcutaneous; PO: per os.

2.3. Atovaquone and azithromycin

Atovaquone is a synthetic hydroxy-1,4-naphthoquinone antiprotozoal agent. The hydroxynaphthoquinones including atovaquone are considered to selectively block protozoan mitochondrial electron transport causing inhibition of pyrimidine and ATP synthesis (Plumb, 2015; de Oliveira Silva et al., 2016). Resistance to atovaquone associated with irresponsiveness to treatment was reported in *B. gibsoni* infected dogs from Japan and Taiwan, and results from mutations in the parasite's cytochrome *b* gene apparently affecting atovaquone's site of action (Matsuu et al., 2006, Sakuma et al., 2009; Iguchi et al., 2012; Liu et al., 2016). Cytochrome *b* is part of a mitochondrial electron transport complex and single point mutations in its gene in *Plasmodium falciparum* and *Plasmodium berghei* are also associated with resistance to atovaquone in the treatment of human malaria (Siregar et al., 2008). The source of modification rendering resistance against atovaquone in resistant *B. gibsoni* was found to be mutations causing the substitution of the amino acid methionine with isoleucine in the cytochrome *b* gene termed the M121I mutation (Matsuu et al., 2006; Lin et al., 2012). An in vitro screening of growth inhibition of *B. gibsoni* with 15 different drugs found atovaquone to be the most effective against the Aomori strain of the parasite (Matsuu et al., 2008).

Azithromycin (Azenil®, Zithromax®, Zmax®) is a macrolide antibiotic that binds to the 50S subunit of the prokaryote ribosome and inhibits the translation of mRNA and bacterial protein synthesis. However, it also has antiprotozoal effects by specifically acting on apicoplasts, which are relict non-photosynthetic plastid organelles containing a limited genome and found in apicomplexan parasites (Chakraborty, 2016) including *Babesia* spp. (Wang et al., 2017). Azithromycin has been shown to cause a delayed death effect in *P. falciparum* by acting on the parasite's apicoplast (Dahl and Rosenthal, 2007), and to also have activity against *Toxoplasma gondii* (Değerli et al., 2003).

Atovaquone is administered at the extra-label dose of 13.3 mg/kg per-os (PO) q8h with azithromycin at 10 mg/kg PO once daily both for 10 days for the treatment of *B. gibsoni*, *B. conradae* and *B. vulpes* (*B. microti*-like) infections (Di Cicco et al., 2012; Checa et al., 2017). Atovaquone is available as an oral tablet (Mepron®). A tablet containing atovaquone and proguanil HCl (Malarone®) is also available and indicated for human malaria treatment and prophylaxis; however, this combination was reported to cause gastrointestinal adverse effects in dogs due to the proguanil component and should be avoided. There are currently no known adverse effects for atovaquone in dogs. The combination of atovaquone with azithromycin is also used for the treatment of infection with the piroplasm *Cytauxzoon felis* in cats (Cohn et al., 2011).

2.4. Buparvaquone and azithromycin

Buparvaquone is a hydroxynaphthoquinone antiprotozoal drug related to atovaquone and parvaquone. Buparvaquone (Butalex®, Vetolex®) is used in the treatment of bovine theileriosis (Wilkie et al., 1998) and has also been found to have a transitory effect against *Theileria equi* infections in equids (Zaugg and Lane, 1989, 1992). It is also used to treat *B. vulpes* infection in dogs at an off-label dose of 5 mg/kg IM twice 48 h apart, in combination with azithromycin at 10 mg/kg PO once daily for 10 days (Checa et al., 2017).

Buparvaquone's mode of action on piroplasms is probably comparable to atovaquone's and similarly to the mechanism of *B. gibsoni* resistance to atovaquone, point mutations in the *Theileria annulata* cytochorome b gene have been associated with resistance to buparvaquone treatment of bovine theileriosis in cattle (Sharifiyazdi et al., 2012; Mhadhbi et al., 2015).

2.5. Other drugs

Several other antiprotozoal drugs such as phenamidine, pentamidine, parvaquone, artemisinin derivatives and antibiotics with some anti-protozoal activity such as doxycycline, minocycline, clindamycin, enrofloxacin and metronidazole have been evaluated for the treatment of canine babesiosis with various levels of efficacy (Wulansari et al., 2003; Matsuu et al., 2008; Lin and Huang, 2010; Lin et al., 2012; Iguchi et al., 2015). Phenamidine is administered to dogs at 15–20 mg/kg SC with an optional second administration after 48 h. It induces side effect that include hypotension, tachyucardia, vomiting and pain at the injection site and is currently not registered in Europe for veterinary use (http://www.esccap.org/uploads/docs/ih38c2d6_ESCCAP_Guidelines_GL5_01Oct2012.pdf). Combinations of three drugs such as clindamycin, imidocarb dipropionate and diminazene aceturate have shown advantages over atovaquone and azithromycin treatments in the therapy of *B. gibsoni* (Lin et al., 2012) and it is likely that a synergistic effect due to interaction of several drugs acting on different parasite targets is beneficial in suppressing parasite growth and acitivity.

3. Current treatment of canine *Babesia* spp.

3.1. Babesia canis

Babesia canis (*B. canis canis*) is a large *Babesia* sp. prevalent mostly in central and northern Europe and transmitted by the tick *Dermacentor reticulatus* (Solano-Gallego et al., 2016). Canine *B. canis* infections have also emerged recently in the United Kingdom (de Marco et al., 2017). *Babesia canis* causes a moderate to severe disease, which frequently has an acute onset (Zygner et al., 2014; Eichenberger et al., 2016). Previous literature has often regarded *B. canis*, *B. vogeli* and *B. rossi* as one species, or as subspecies of *B. canis* (Uilenberg et al., 1989; Uilenberg 2006), and, therefore, older studies on treatment of large canine *Babesia* spp. have often used the term *B. canis* when relating to the species currently referred to as *B. vogeli*, *B. canis* and *B. rossi*. This must be recognized when reading the older literature on treatment of canine babesiosis. Studies that describe treatment of true *B. canis* infections are usually from central and northern Europe. Canine *B. canis* infection is

commonly treated with imidocarb dipropionate (Matjila et al., 2005; Bajer et al., 2014; Adaszek et al., 2015; Eichenberger et al., 2016) and the European Scientific Counsel Companion Animal Parasites (ESCCAP) recommended its use for treatment of *B. canis* in its 2012 guidelines for control of vector-borne diseases in dogs and cats (http://www.esccap.org/uploads/docs/ih38c2d6_ESCCAP_Guidelines_GL5_01Oct2012.pdf). Treatment of *B. canis* with diminazene aceturate or both imidocarb dipropionate and diminaze aceturate is also effective (Beugnet et al., 2014).

3.2. Babesia rossi

Babesia rossi (*B. canis rossi*) is a large form *Babesia* sp. reported from South Africa as well from other sub-Saharan African countries including Sudan and Nigeria (Oyamada et al., 2005; Penzhorn, 2011; Kamani et al., 2013). Its tick vectors are *Haemophysalis eliptica* and *Haemophysalis leachi*, which were previously considered as one species (Apanaskevich et al., 2007). *Babesia rossi* is regarded as the most virulent large *Babesia* sp. that affects dogs and most of the studies on its pathogenicity and epidemiology are from South Africa (Goddard et al., 2016; Rautenbach et al., 2017).

Babesia rossi infections are usually treated with diminazene aceturate in Africa, although adequate response has also been described to treatment with imidocarb dipropionate in South Africa (Collett, 2000), as well as in a dog imported from that country to the USA one week before presentation due to illness at a Texas veterinary clinic and successful treatment with imidocarb dipropionate (Allison et al., 2011).

3.3. Babesia vogeli

Babesia vogeli (*B. canis vogeli*) is a large *Babesia* sp. transmitted by *Rhipicephalus sanguineus* sensu lato ticks and found mostly in tropical and subtropical regions including the Mediterranean basin, the Middle East, Asia, Australia, and South, Central and North America (Solano-Gallego and Baneth, 2011). It can cause subclinical infection or mild to moderate disease. It may cause a severe illness in young pups and in greyhounds. *Babesia vogeli*-infected dogs often present with co-infections, frequently with *Ehrlichia canis* or *Hepatozoon canis*, which are also transmitted by the same tick vector (Singla et al., 2016). The treatment of choice recommended for canine *B. vogeli* infection by the Companion Animal Parasite Council (https://www.capcvet.org/guidelines/babesia/) in the USA is with imidocarb dipropionate.

3.4. Unnamed large Babesia

A currently unnamed large form *Babesia* sp. was detected for the first time in a dog treated by chemotherapy for lymphoma (Birkenheuer et al., 2004b). Seven immunocompromised dogs infected with this pathogen were subsequently reported in the eastern USA (Sikorski et al., 2010) and another dog from Texas (Holman et al., 2009). All the dogs infected with this species 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 *Babesia* have revealed a unique sequence that shared a 93.9% identity with *Babesia bigemina* (Birkenheuer et al., 2004b). Treatment of most of the dogs infected with the unnamed large *Babesia* with imidocarb diprpionate at the recommended dose was successful in relieving most of the clinical signs and hematological abnormalities associated with babesiosis. Nevertheless, elimination of the parasite was not achieved in some dogs, possibly due to other causes of immune-suppression (Holman et al., 2009; Sikorski et al., 2010).

3.5. Babesia gibsoni

Babesia gibsoni is a small form *Babesia* that is endemic in Southeast Asia and Japan and appears to have spread from there to other continents including Australia, Europe, and North and South America. It is a common and often subclinical cause of infection in Pitt Bull Terriers, which may also inflict a severe disease in this breed as well as in other dog breeds. It is transmitted by the ticks *Haemaphysalis longicornis* and possibly by *Haemaphysalis bispinosa* and *R. sanguineus* s.l. There is also evidence for its direct transmission by dog bites (Birkenheuer et al., 2005; Jefferies et al., 2007a; Yeagley et al., 2009).

Imidocarb dipropionate and diminazene aceturate are considered ineffective for the treatment of *B. gibsoni* infections and treatment with the combination of atovaquone and azithromycin is the current treatment of choice for this infection, although suppressing parasite replication by this combination may not be associated with parasite clearance (Jefferies et al., 2007b; Lin et al., 2012, Kirk et al., 2017).

Mutations in the parasite's cytochrome *b* gene associated with resistance formation to atovaquone in some *B. gibsoni* strains present a clinical problem, as this resistance is widespread and reported from multiple areas mainly in Japan and Taiwan (Matsuu et al., 2006, Sakuma et al., 2009; Iguchi et al., 2012, Liu et al., 2016). A study in dogs comparing the combination of atovaquone and azithromycin with a triple drug combination of clindamycin, diminazene aceturate and imidocarb dipropionate found that this triple combination had higher recovery and lower relapse rates, but longer treatment duration and slower reduction in parasitemia than atovaquone with azithromycin. The clindamycin, diminazene aceturate and imidocarb dipropionate triple combination was therefore recommended for treatment of suspected atovaquone resistant *B. gibsoni* infections (Lin et al., 2012).

3.6. Babesia conradae

Babesia conradae is a small *Babesia* reported in dogs from California. It was initially thought to be a strain of *B. gibsoni* and later found to be a genetically distinct species, which causes a severe and potentially fatal disease in dogs. The tick vector of *B. conradae* has not been described to date (Kjemtrup and Conrad, 2006). *Babesia conradae*, like other small babesial species, appears not to be susceptible to imidocarb dipropionate; nevertheless, treatment with a combination of atovaquone and azithromycin was shown to be effective in curing infected dogs and eliminating the parasite as indicated by repeated PCR of treated dogs (Di Cicco et al., 2012).

3.7. Babesia vulpes

Babesia vulpes, described initially as *T. annae* and also termed *B. microti*-like, *Babesia* cf. *microti* and *Babesia annae*, is a small species of *Babesia* that infects dogs (Zahler et al., 2000; Baneth et al., 2015). It was described from a dog that originated in northern Spain and has subsequently been found in other European countries and in North America (Yeagley et al., 2009; Miró et al., 2015). *Babesia vulpes* is a common cause of infection in wild red-foxes (*Vulpes vulpes*). Although it has been speculated that it is transmitted by the hedgehog tick *Ixodes hexagonus* and by *Dermacentor reticulatus* (Camacho et al., 2003; Hodžić et al., 2017), there is currently no definitive evidence of its transmission by these tick species or other species.

A study of 59 dogs naturally infected with *B. vulpes* in Spain compared treatment with imidocarb dipropionate, to atovaquone and azithromycin, or buparvaquone and azithromycin (Checa et al., 2017). Forty-seven percent of the dogs treated with imidocarb dipropionate had a clinical relapse within 1 year indicating that treatment with this drug was insufficiently effective. Nevertheless, despite clinical cure of most dogs treated with either combination therapies within 90 days of treatment initiation, parasitemia detected by PCR persisted after one year in 50% of the dogs treated with atovaquone and azithromycin, 60% of those treated with buparvaquone and azithromycin, and 73% of those treated with imidocarb. Thus, none of the treatments was successful in elimination of *B. vulpes*.

4. Conclusions

Treatment of canine babesiosis should be addressed with particular attention to the specific response of every *Babesia* spp. to different drugs and, considering the characteristics of strains within species, in particular drugs resistant strains. The individual clinical status of the affected dog, its age and whether it is immune-depressed should also be deliberated. A limited number of effective drugs are currently available for antibabesial treatment of dogs, and there are clearly differences in drug susceptibilities between large and small *Babesia* spp. and within that group of species. There are major obstacles are in the treatment of small *Babesia* spp., which are frequently not eliminated despite treatment and thus treated dogs may remain as chronic carries capable of transmitting infection further and relapsing with clinical disease. Treated dogs should be followed up and monitored for persistent infection and parasitemia by blood smear examination and PCR. Furthermore, additional and new drugs, and the synergistic effects of current drug combinations against *Babesia* infection should be studied to find effective treatments for canine babesiosis.

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