THE PALE & THE BLEEDING:
IMMUNE & INFECTIOUS BLOOD DISORDERS

Babesiosis, Ehrlichiosis & Immune-Mediated Damage to Red Cells & Platelets

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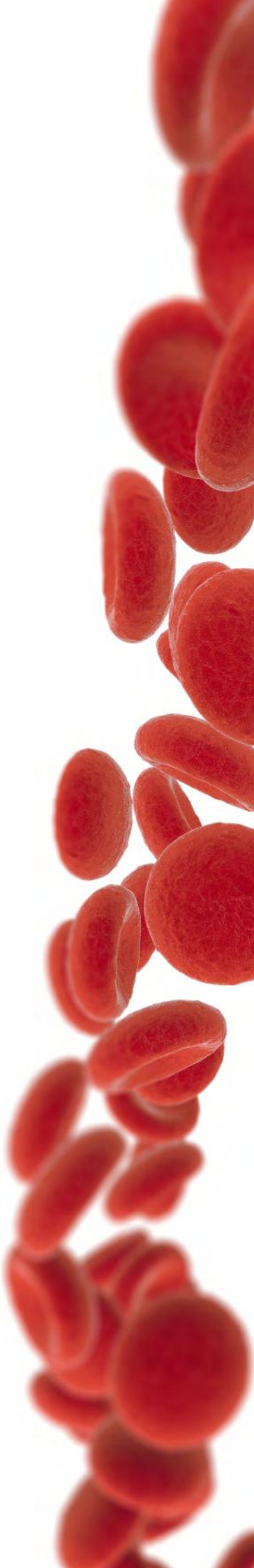


TICK-BORNE DISEASES

Vector-borne diseases of pets, and in particular tick-brone diseases transmitted by ixodid tick cause severe and potentially fatal diseases in dogs. Although there are only a few publications on canine vector-borne diseases from Hong Kong, it is apparent that infections with Ehrlichia canis and Babesia gibsoni are prevalent in the dog population and that there is also considerable awareness of these diseases and their prevention among dog owners. Confronting tick-borne infections is a major part of the veterinary practitioners routine in areas of high endemicity and it is important to keep updated with progress on the diagnosis, treatment and prevention of these diseases.

CANINE EHRLICHIOSIS

INTRODUCTION

Ehrlichia canis is the causative agent of canine monocytic ehrlichiosis which is an important canine infectious disease in Africa, Asia, America, and Europe. Ehrlichia canis is transmitted by the three-host tick Rhipicephalus sanguineus. A recent study has shown that transmission of E. canis by R. sanguineus starts within 3 hours after the tick´s attachment to the canine host. The pathogenesis of the disease involves an incubation period of 8-20 days, followed by three consecutive phases: an acute phase which lasts 1-4 weeks, a subclinical phase which may last from months to years, and a chronic phase. Not all infected dogs develop the chronic severe form of the disease and the conditions that lead to the development of this stage are assumed to be associated with individual susceptibility and breed predisposition. Ehrlichia canis can be transmitted by blood transfusion and it is recommended to screen for its presence in the blood of donor dogs.

THE THREE STAGES OF **CANINE EHRLICHIOSIS**

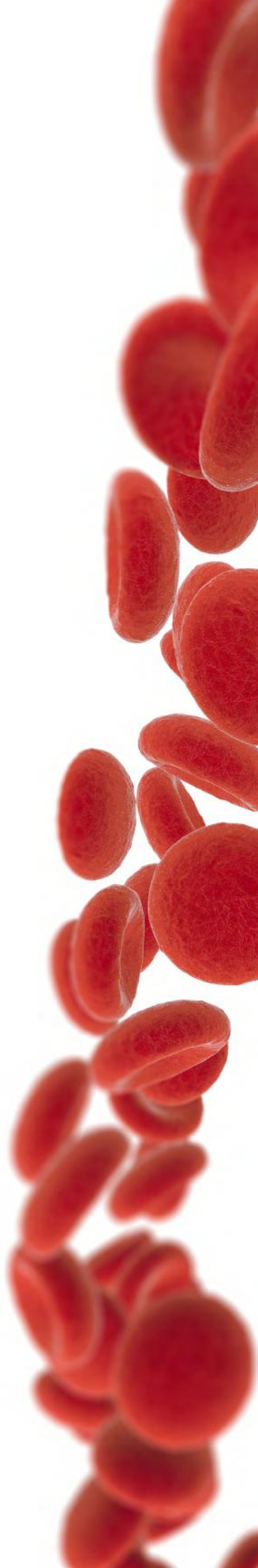
INCUBATION PERIOD OF 8-20 DAYS

ACUTE PHASE

SUB-CLINICAL PHASE

CHRONIC PHASE

Death due to sepsis or anemia



CLINICAL FINDINGS IN CANINE EHRLICHIOSIS

The most frequently reported clinical signs of canine monocytic erhlichiosis are lethargy, anorexia, fever, lymphadenomegaly, splenomegaly and hemorrhages, mainly petechiae, ecchymoses and epistaxis. Ocular manifestations of canine ehrlichisosis include anterior uveitis, keratoconjuctivitis, hyphema, glaucoma, chorioretinitis and retinal detachment. Polyarthritis and polymyositis have also been described in E. canis infection. The neurological abnormalities found in canine ehrlichiosis are associated with vasculitis, meningoencephalitis, and lymphocytic infiltration of the central and peripheral nervous system or hemorrhages. Ehrlichia canis infection has been termed the «silent killer» as its infection is often not apparent during the early and sub-clinical stages of infection, and when the disease is diagnosed in the chronic stage, it may be too late to save the canine patient, as treatment may not be helpful in reversing the severe pancytopenia caused by bone marrow suppression associated with this disease.

Laboratory abnormalities in canine monocytic enrlichiosis include hematologic and serum biochemistry changes. Thrombocytopenia is the most frequent hematological abnormality occurring in more than 90% of cases. Anemia, usually non-regenerative normocytic and

normochromic, is another common finding in this disease. In addition, mild to severe leucopenia is a frequent abnormality.

Hyperglobulinemia, hypoalbuminemia and mild elevation of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities are frequently reported in ehrlichiosis. Dogs in the chronic severe stage of the disease may develop severe pancytopenia as their bone marrow becomes hypocellular and the prognosis of these chronically ill dogs is grave.

Immune-mediated responses play a major role in the pathogenesis of E. canis infection. Anti-platelets antibodies have been demonstrated less than a week after experimental E. canis infection of dogs. Platelet aggregation abnormalities, anti-nuclear antibodies, RBC autoagglutination with positive coombs› test, and circulating immune-complexes have been described in infected dogs and are associated with the disease process.

The decrease in platelets during canine ehrlichiosis is a result of several mechanisms. These mechanisms include increased consumption with vascular endothelial changes, platelet sequestration and pooling in the spleen, thrombophagocytosis with immunological destruction, a decrease in the half life time of circulating platelets due to opsonization with antibodies, and production impairment due to bone marrow destruction and hypocellularity. In addition to the decrease in circulating platelet number, platelets dysfunction (thrombocytopathy) has also been implicated as an additional factor contributing to lack of platelet functionality in canine monocytic ehrlichiosis.

DIAGNOSIS OF CANINE EHRLICHIOSIS

DIAGNOSIS OF CANINE EHRLICHIOSIS

The diagnosis of canine monocytic ehrlichiosis includes evaluation of the hemogram and serum biochemistry panel. Specific diagnosis of infection includes:

(1) PCR: Detection of the presence of E. canis DNA by PCR is highly sensitive and specific and has become the most useful diagnostic test for the confirmation of canine monocytic ehrlichiosis. Several conventional and real-time PCR protocols have been described for E. canis and the assay can be performed on blood or tissue including the spleen and bone.

(2) Serology: Anti-E. canis antibodies persist long after recovery from the disease. Serum antibodies are thought not to be protective or play an important role in eliminating this intracellular infection. Serology is indicative of exposure to E. canis and may often be helpful in ruling out progressive infection. Antibodies may not be detectable during the early stage of infection. Furthermore, seropositive dogs with previous exposure to the pathogen may also present in the clinic due to other urgent disease conditions.

(3) Cytology: E. canis morulae found in monocytes and macrophages are a “microcolony” of bacteria surrounded by a membranous vacuole. Morulae may contain 100 or more ehrlichiae organisms. The detection of morulae in monocytes in stained blood smears is rare and cannot serve as a main diagnostic option.

METHODS FOR THE DETECTION OF **CANINE EHRLICHIOSIS**

- **Microscopic detection of organisms (morulae)**
- **Serology**
- **PCR**

TREATMENT OF **CANINE EHRLICHIOSIS**

Ehrlichia canis is susceptible to doxycycline which is highly efficient in clearing rickettsemia in acute cases of E. canis infection. Clinical recovery is noticed within 48-72 hours, yet treatment should be continued for 3 weeks, as some dogs may remain carriers when shorter treatments are applied. Treatment with the injectable drug imidocarb dipropionate has been shown to be ineffective in totally eliminating E. canis. However, it is often used in combination with doxycyline when Babesia co-infection is suspected.

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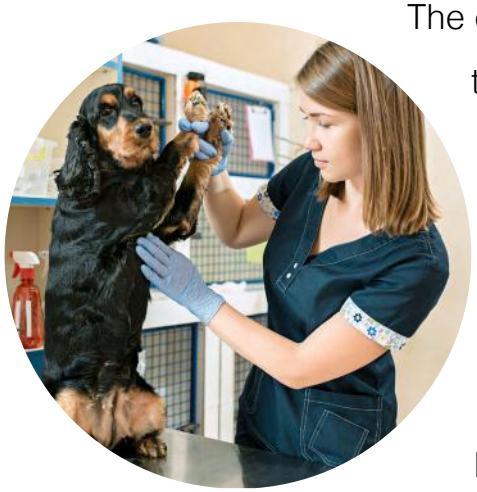
PREVENTION OF **CANINE EHRLICHIOSIS**



The control of tick infestation by topical treatment with acaricidals and environmental eradication of ticks is recommended for the prevention of E. canis. No vaccines for the disease are currently available, however, a candidate attenuated live vaccine has been recently evaluated for preventing the disease.

CANINE ANAPLASMA PLATYS INFECTION

Anaplasma platys was first identified in 1978 in Florida. It is a Gram-negative, obligate intracellular bacterium belonging to the family Anaplasmataceae and closely related to Anaplasma phagocytophilum and Ananplasma marginale. Anaplasma platys infects canine platelets and causes a disease commonly recognized as infectious canine cyclic thrombocytopenia. The presumed natural vector of A. platys is the tick R. sanguineus and DNA of this bacterium has been reported from this tick species in several countries, however, experimental infection studies have not demonstrated transmission by R. sanguineus conclusively. Like E. canis, A. platys may also be accidentally transmitted by blood transfusion.

CLINICAL FINDINGS IN ANAPLASMA PLATYS INFECTION

Anaplasma platys causes a cyclic thrombocytopenia that could result in bleeding, including petechiae and ecchymoses, although most infected dogs are probably able to control infection without demonstrating clinical signs. Bacteremia and thrombocytopenia occur in cycles of approximately 10 to 14 days. The clinical findings associated with infection according to some published studies include: anorexia, lethargy, fever, weight loss, lymphadenomegaly, petechiae and ecchymoses, thrombocytopenia and anemia. Other studies have described asymptomatic natural infection associated with A. platys. Infected dogs are frequently co-infected with E. canis, and an experimental infection of dogs with both agents has shown that dogs with simultaneous infections had more severe clinical manifestations than those infected only with one agent.

DIAGNOSIS OF **ANAPLASMA** PLATYS INFECTION

Detection of A. platys morulae in canine platelets can be made upon examination of Giemsa-stained blood smear by microscopy, however, confirmation of infection should be made by specific PCR.

TREATMENT OF **ANAPLASMA** PLATYS INFECTION

Anaplasma platys is susceptible to doxycycline with at the same dose and duration used for E. canis treatment.

Further reading on canine ehrlichiosis and anaplasmosis

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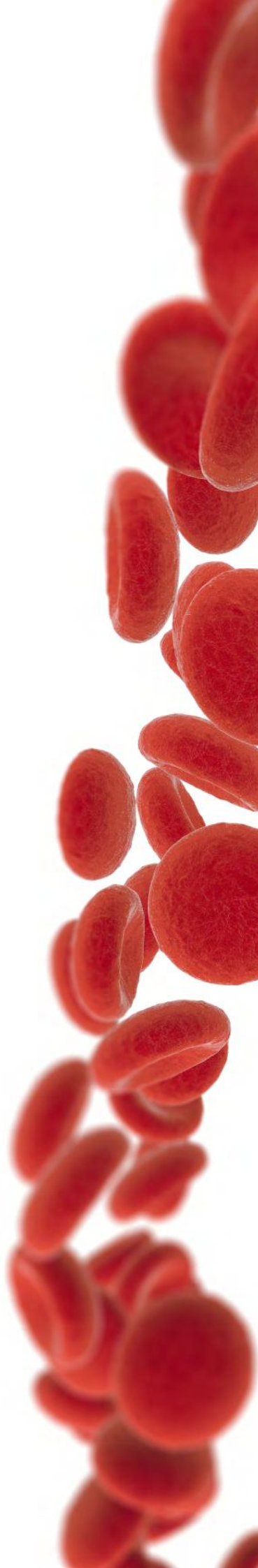


CANINE BABESIOSIS
WITH EMPHASIS ON BABESIA GIBSONI INFECTION

INTRODUCTION

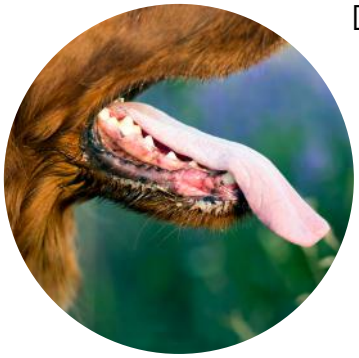
Babesiosis is caused by protozoal parasites that infect erythrocytes and cause anemia. Babesia species are tick-borne apicomplexan parasites that infect a variety of domestic and wild animals and may cause moderate to severe disease. Babesiosis has a worldwide distribution and global importance. Hemolytic anemia with erythrocyte destruction and a systemic inflammatory response account for most of the clinical signs observed in canine and feline babesiosis.

CANINE **BABESIOSIS**

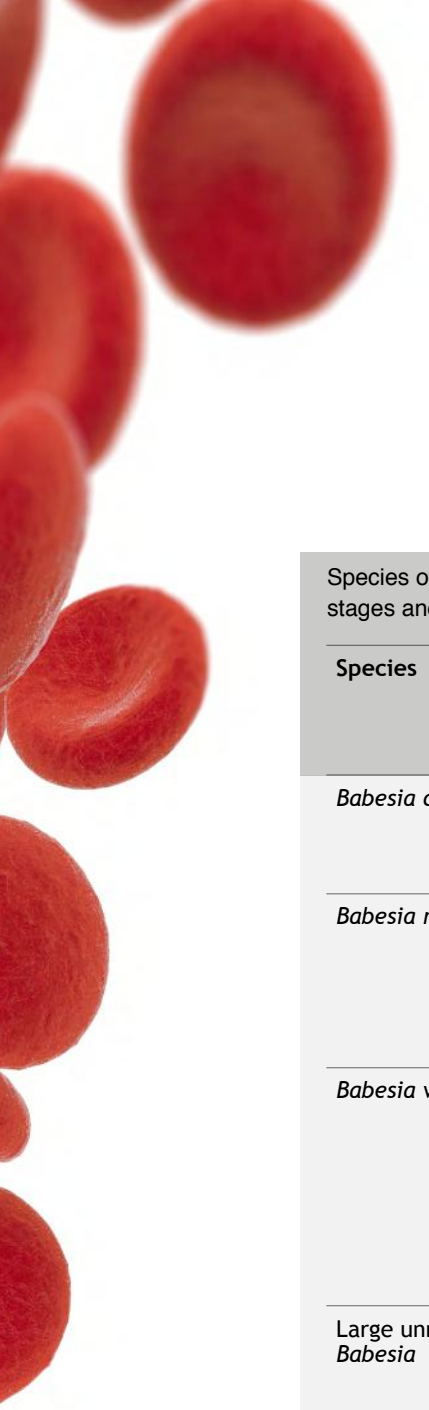
Babesia infection was identified in the past based on the morphologic appearance of the parasite in erythrocytes. All large forms of canine Babesia (2.5–5.0 µm) were designated Babesia canis, whereas all the small forms (1.0–2.5 µm) were considered as Babesia gibsoni. However, the development of molecular methods have demonstrated that more piroplasmid species infect dogs and cause different diseases. Babesia rossi, B. canis and B. vogeli previously considered as subspecies are identical morphologically but differ in the severity of clinical manifestations which they cause, their tick vectors, genetic characteristics, and geographic distributions, and are therefore currently considered separate species. Another yet unnamed large Babesia sp. most closely related to B. bigemina was found to infect immunocompromised dogs in North America. The small Babesia spp. that infect dogs include B. gibsoni, B. conradae described from California, and the B. vulpes (synonym B. microtri-like; Theileria annae). None of the Babesia species that infect dogs has been found to be zoonotic.

The geographical distribution of the causative agents 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 dogs breeds independently of the limitations of vector tick infestation. B. gibsoni is likely transmitted directly from dog to dog via bite wounds, saliva, or ingested blood.

Babesia vogeli and B. gibsoni have wide distributions in both the Old and New World continents, whereas B. rossi has to date been mostly restricted to Africa and B. canis has mostly been reported from Europe. Babesia gibsoni 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. Babesia gibsoni is transmitted by Haemaphysalis longicornis, Haemaphysalis hystricis recently reported as a vector in Taiwan, and perhaps by Rhipicephalus sanguineus.



Dogs are infected when Babesia sporozoites are injected with saliva into the host's skin during the blood meal. The parasites invade the erythrocytes and form ring-shaped trophozoites. The parasite replicates 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 erythrocyte and eventually destroying the cell freeing into the blood to invade more erythrocytes. Ticks feeding on infected blood take up merozoites and sexual parasite development in the tick gut is followed by sporogony in its tissues. The parasite reaches the tick salivary glands or it's



oocytes from which transmission occurs. Babesia spp. are transmitted transstadially from one stage in the tick life cycle to another, and also transovarially through the tick eggs, as shown for some Babesia spp, The transmission of babesiae occurs through the bite of a vector tick. However, B. gibsoni infection has also been demonstrated to be transmitted via blood transfusion and transplacentally.

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 x 5 (large forms)	Imidocarb dipropionate
Babesia rossi	Southern Africa, Nigeria, Sudan	*Haemaphysalis elliptica* *Haemaphysalis leachi*	2 x 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 x 4.5 (large forms)	Imidocarb dipropionate
Large unnamed *Babesia*	Eastern United States	Unknown	2 x 6 (large forms)	Imidocarb dipropionate
Babesia gibsoni	Southeast Asia, United States, Australia, Europe	*Haemaphysalis longicornis* *Haemaphysalis bispinosa*? *R.. sanguineus* s.l.?*	1 x 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 x 2.5 (small forms)	Atovaquone and azithromycin; bupravaquone and azithromycin

** ? refers to a suspected vector.*

It is important to remark that clinical findings are variable depending on the Babesia species infecting dogs. In general, hemolytic anemia and the systemic inflammatory response syndrome leading to multiple-organ dysfunction syndrome are responsible for most of the clinical signs observed in canine babesiosis. Hemolysis may result in hemoglobinemia, hemoglobinuria, bilirubinemia and bilirubinuria.

Thrombocytopenia is consistently observed in babesiosis and may be caused by immune mechanisms, splenic sequestration or coagulatory consumption of platelets from hemolytic or vascular injury. Immune mediated thrombocytopenia has been demonstrated in experimental canine babesiosis caused by B. gibsoni.

Tissue hypoxia is found in severe canine babesiosis. It is caused by anemia, hypotensive shock, vascular stasis by sludging of erythrocytes, excessive endogenous production of carbon monoxide, and parasitic damage to hemoglobin. The central nervous system, kidney, and muscle are the organs most affected by tissue hypoxia. Tissue hypoxia, hypertensive shock, multiple organ dysfunction and potential mortality have been documented mostly in association with B. rossi and B. canis infections. Young pups and immunocompromised adult dogs, such as dogs with hyperadrenocorticism or treated with immunosuppressive therapy, may suffer a severe disease with B. vogeli infection.

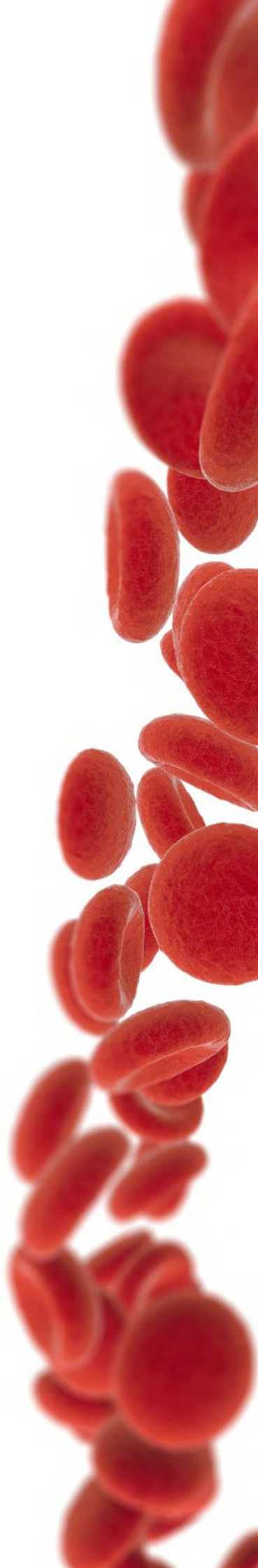
The spleen has an important function in controlling babesiosis. Experimentally infected splenectomized dogs rapidly develop parasitaemia and clinical disease and may reach high parasitaemia levels. Splenectomy has also been associated with natural canine and human babesiosis.

DIAGNOSIS OF BABESIOSIS

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. A fresh smear is recommended for the accurate diagnosis of infection. Erythrophagocytosis with infected erythrocytes may be found in blood smears from infected dogs. The use of molecular diagnostic assays such as PCR is indicative in cases of low parasitemia including suspected carrier dogs or chronically infected animals as well as for speciation.

TREATMENT OF CANINE BABESIOSIS

Large Babesia spp. are commonly treated with imidocarb dipropionate with good clinical response while small Babesia spp. appear to be more difficult to treat and resistant to the conventional drugs that are effective against the large babesial spp. (Table 4.2). Diminazene aceturate used for treatment of both large and



small babesial spp. infections should be used cautiously as it has a relatively small dose safety margin with a large inter-individual pharmacokinetic variation. Babesia gibsoni infection is often resistant to imidocarb dipropionate and diminazene aceturate and an alternative therapy with the combination of the anti-malarial atovaquone and the macrolide azithromycin has been recommended for this infection. However, complete clinical and parasitological cure are not commonly achieved in dogs treated for small babesial spp. infections and clinical relapses may occur. Medical management of infection may require supportive treatments including blood transfusions, intravenous fluids, and the use of anti- inflammatory drugs.

Mutations in the parasite's cytochrome b gene are associated with resistance formation to atovaquone in some B. gibsoni strains. These present a serious clinical problem, as this resistance is widespread and reported from multiple areas mainly in Japan and Taiwan. 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.

The main 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, 2016)
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 48h 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)

Further reading on canine babesiosis

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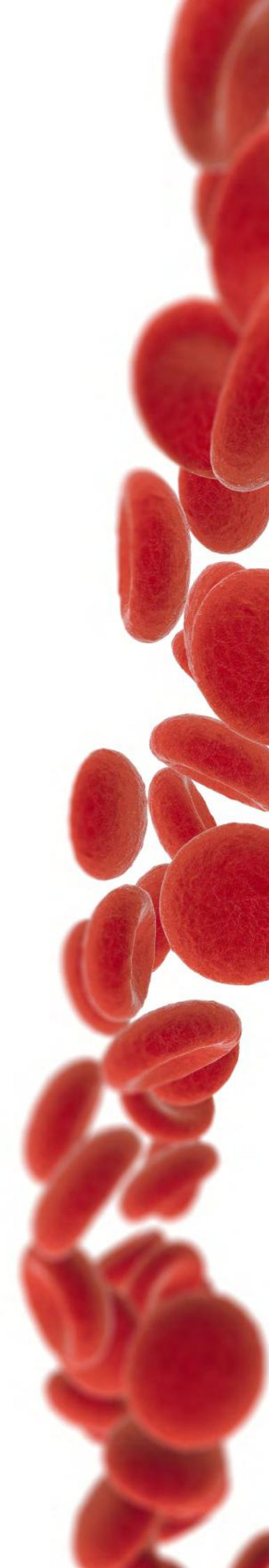

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