CASE REPORT
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COMBINATION THERAPY WITH DOXYCYCLINE, CLINDAMYCIN AND METRONIDAZOLE FOR *BABESIA GIBSONI IN* A DOG AND ITS COMPLETE REMISSION

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ABSTRACT

The *Babesia* genus includes intracellular pathogens that can infect the erythrocytes of various species, including humans, dogs, cats, horses, and wild mammals. A five-year-old female Labrador Retriever was brought in with symptoms such as reduced appetite, fever, laboured breathing, lethargy, and dark yellow urine. Clinical examination showed an elevated body temperature (104.5ºF), pale mucous membranes, a severe tick infestation, and enlarged prescapular lymph nodes. Routine blood tests, serum biochemistry, peripheral blood smear examination, and PCR were conducted. Haematological results revealed severe regenerative anaemia, and the blood smear displayed numerous signet ring-shaped *Babesia gibsoni* parasites (+++) in the red blood cells. The diagnosis was confirmed through Real-Time Polymerase Chain Reaction (PCR). The dog was treated with a combination of doxycycline, clindamycin, and metronidazole. After ten days, the dog showed significant clinical improvement, and follow-up blood smears were negative for *Babesia* parasites. This case demonstrated the successful use of triple drug therapy in completely eliminating a natural infection caused by *Babesia gibsoni* in a dog.

Keywords: Babesia, Doxycycline, Haemoprotozoan, Triple drug therapy

INTRODUCTION

Babesia species are haemoparasites found worldwide that cause disease in various mammal species (Onyiche *et al.,* 2021). Canine babesiosis is a serious tick-borne disease affecting dogs, caused by intraerythrocytic protozoa of the *Babesia* genus, and is a significant health threat in dogs globally (Mittal *et al.,* 2019). First identified by Babes in 1888 as a cause of haemolytic anaemia in cattle, canine babesiosis is now widespread and endemic

in regions such as North America, North Africa, East Africa, the Middle East, and Asia (Birkenheuer *et al*., 2005). *Babesia* species are intra-erythrocytic parasites transmitted by ixodid ticks or directly through blood transfusions or bite wounds. The prevalence of the disease depends on geography and the presence of tick vectors (Otsuka, 1974). In dogs, large *Babesia* species include *Babesia canis*, where intra-erythrocytic merozoites measure 3–5 micrometres (about half the size of a red blood cell), while smaller species like *Babesia gibsoni*, *B. conradae*, and *B. vulpes* have merozoites measuring 1–3 micrometres (Carret *et al*., 1999). The size of *Babesia* parasites relative to the erythrocyte (approximately 7 micrometres) is helpful for identifying the specific species affecting the dog (Karasová *et al*., 2022). Transmission can also occur via blood transfusions and direct inoculation during fights between infected animals. The two most common species that infect dogs are *Babesia canis* and *Babesia gibsoni*, both transmitted by ixodid ticks (Sunitha *et al*., 2011). In some cases, dogs may carry the infection without showing significant symptoms, but severe cases can lead to fever, haemolytic anaemia, thrombocytopenia, and even death if untreated (Birkenheuer *et al*., 1999). Thrombocytopenia in dogs may result from immune-mediated platelet destruction, platelet sequestration in the spleen, or consumptive coagulopathy, such as disseminated intravascular coagulation (Boozer and Macintire, 2003).

Thrombocytopenia in canine babesiosis is less likely to be due to decreased platelet production, as increased mean platelet volume (MPV) suggests regeneration from the bone marrow (Preena *et al*., 2021). Pseudo-thrombocytopenia, caused by platelet clumping or platelets adhering to red blood cells, has been observed in conditions such as falciparum malaria (Zvorc *et al*., 2010; Goddard *et al*., 2015). Conversely, in cases of canine babesiosis, the increase in large, activated platelets—reflected by elevated MPV and mean platelet mass—may preserve functional platelet mass, reducing the risk of bleeding despite severe thrombocytopenia. These large, immature platelets are considered more functionally active, with a lower threshold for aggregation and release (Goddard *et al*., 2015).

In a study of 45 dogs exhibiting clinical signs like fever, lymphadenopathy, and anorexia, Giemsa-stained blood smears revealed intra-erythrocytic *Babesia gibsoni* parasites, appearing as single or multiple signet-ring forms (Soulsby, 1982). Egege *et al*. (2008) found a higher incidence of the disease in dogs aged one to three years, likely due to reduced maternal immunity and frequent tick exposure. Anaemia was

one of the most common findings in dogs with babesiosis (Vishnurahav *et al*., 2017), with low erythrocyte counts linked to factors such as mechanical damage to red blood cells, splenic removal of infected RBCs, and immune-mediated erythrocyte destruction (Meinkoth *et al*., 2002). Dogs with suppressed immune systems may progress from asymptomatic carriers to clinical disease. The combination of atovaquone and azithromycin (AA) is currently the preferred treatment for *Babesia gibsoni* infections in many regions, although there have been reports of treatment failures due to mutations in the *Babesia* cytb gene, which may cause resistance to atovaquone (Sakuma *et al*., 2009).

CASE HISTORY AND OBSERVATION

A five-year-old female Labrador retriever was presented with symptoms of reduced appetite, fever, difficulty breathing, lethargy, and dark yellow urine. On clinical examination, the dog had an elevated body temperature (104.5ºF), pale mucous membranes, a severe tick infestation, and prescapular lymphadenopathy. A physical exam also revealed slight splenomegaly. Routine haematological tests and serum biochemistry were conducted (Table 1). Key findings from the haematology included anisocytosis, polychromasia, nucleated red blood cells, mild thrombocytopenia, and neutrophilic leucocytosis, with a left shift due to a marked systemic inflammatory response in the early stages of pathogenesis. Examination of a Leishman-Giemsa-stained peripheral blood smear showed signet ring-shaped piroplasms inside red blood cells, indicating the presence of *Babesia gibsoni* (Fig 1). The serum biochemistry results were inconclusive. A whole blood sample was submitted for PCR testing for common canine haemoprotozoan infections, including *Babesia*, *Ehrlichia*, and *Mycoplasma*. The PCR test confirmed the presence of *Babesia gibsoni* DNA, leading

Table 1 : Haematological and Biochemical findings in the dog

PARAMETERS	Day 1	Day 10	Day 30
HGB (g/dL)	7.5	9.2	12.89
HCT (%)	21.9	25.63	39.25
WBC (x10^3/microL)	26700	20100	16352
RBC (x 10^6/microL)	2.29	3.15	5.79
PLT (no/microL)	175	257	376
RDW	19.3	17.3	18.25
Neutrophils (%)	86	75	79
Creatinine (mg/dL)	1.1	0.89	1.12
ALT (IU/L)	85	96	78

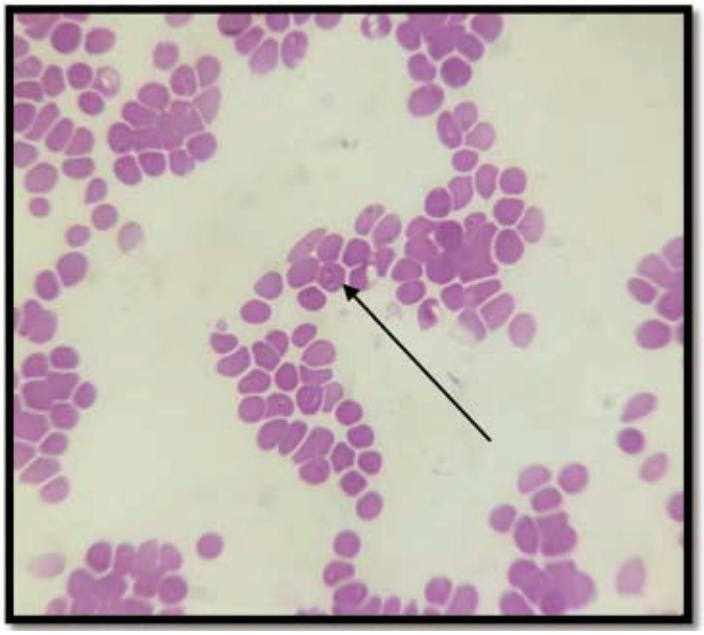

Fig.1. Blood smear (100 X, Leishman- Giemsa) *Babesia gibsoni* - signet ring shaped piroplasms

to a diagnosis of canine babesiosis caused by *Babesia gibsoni*. A saline agglutination test was performed to rule out immune-mediated haemolytic anaemia, and serum biochemistry was repeated every 30 days for three months to confirm the complete clearance of parasitaemia, which was verified by PCR.

TREATMENT AND DISCUSSION

Atovaquone and azithromycin (AA) are currently regarded as the primary treatment for *Babesia gibsoni* infections in most regions. However, there have been reports of drug-resistant strains of *B. gibsoni* emerging in cases treated with this regimen (Wulansari *et al*., 2003). Additionally, *B. gibsoni* is often challenging to fully eliminate with standard treatments, and many dogs remain chronic carriers or experience recurrent acute episodes of babesiosis (Schoeman, 2009). A treatment plan using a combination of doxycycline (5 mg/kg twice daily, orally), clindamycin (25 mg/kg twice daily, orally), and metronidazole (20 mg/kg twice daily, orally) for 21 days was prescribed. Nutritional supplements were also administered. This combination therapy successfully reduced parasitaemia levels, and clinical signs significantly improved within 10 days of treatment. PCR analysis of whole blood after 30 days showed no detectable parasitic DNA. The effectiveness of this triple-drug regimen in clearing the parasitic infection in this case is discussed. In conclusion, this case demonstrated that a combination therapy consisting of doxycycline (5 mg/kg twice

daily, orally), clindamycin (25 mg/kg twice daily, orally), and metronidazole (20 mg/kg twice daily, orally) for 21 days is effective in treating *B. gibsoni* infections. The therapy cleared circulating parasites without causing any adverse reactions, and the dog made a full recovery.

Babesiosis is commonly associated with symptoms such as haemolytic anaemia, thrombocytopenia, fever, and splenomegaly, and it can develop into a severe, life-threatening condition. The distribution of *Babesia* species in dogs varies by region, but due to the movement of infected animals, tick vectors, and improved diagnostic capabilities, the geographical range of many *Babesia* species has expanded (Carter and Rolls, 2016). *Babesia gibsoni* is mainly transmitted by ticks of the *Haemaphysalis* species and possibly by *Rhipicephalus sanguineus*. In its original endemic regions, particularly in Asia, the spread of *B. gibsoni* aligns with the distribution of *Haemaphysalis bispinosa*, and tick infestations are a known risk factor, even for non-fighting breeds. The clinical signs of canine babesiosis result primarily from haemolysis - both intravascular and extravascular - along with the removal of healthy erythrocytes by phagocytosis. This occurs due to the increased fragility of non-infected red blood cells and the binding of circulating antigen-antibody complexes to the surface of the red blood cells, leading to their removal by the reticuloendothelial system. Thrombocytopenia in infected dogs may be caused by immune-mediated platelet destruction or consumption due to coagulopathy, though severe reductions in platelet counts rarely lead to bleeding in most cases of *Babesia* infections (Sykes, 2022).

Clindamycin, an antibiotic derived from lincomycin, has been found to stimulate both cellular and humoral immune responses, making it effective against *B. gibsoni* (Wulansari *et al*., 2003). Doxycycline, a tetracycline antibiotic, has shown preventive effects against *Babesia canis* (Vercammen *et al*., 1996), while metronidazole, an antiprotozoal agent, has demonstrated therapeutic efficacy against *B. gibsoni* infections (Fowler *et al*., 1972).

Other potential complications of babesiosis include membranoproliferative glomerulonephritis, which may have an immune-mediated cause. Dogs infected with *B. gibsoni* may develop renal disease and proteinuria (Sykes, 2022). The standard treatment for babesiosis caused by *Babesia gibsoni* includes atovaquone (13.3 mg/kg, three times a day orally) and azithromycin (10 mg/kg once a day orally) for 10 days. The effectiveness of triple drug therapy in eliminating *Babesia gibsoni* parasitaemia has been well-documented, but there are

cases of strains that show resistance to this treatment. The proper dosage and timing of each drug must be closely monitored and discussed further to address issues of drug resistance effectively.

SUMMARY

A female Labrador Retriever aged five years was presented with history of hyporexia, pyrexia, dyspnoea, lethargy and dark yellow urination. Clinical examination revealed high body temperature (104.5ºC), pale mucous membranes, severe tick infestation and prescapular lymphadenopathy. Routine haematology and serum biochemistry panel were performed along with peripheral blood smear examination and PCR. The diagnosis was confirmed by Polymerase Chain Reaction. The present study can be used to evaluate the efficacy of triple drug therapy in natural infection of *Babesia gibsoni* in dog.

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