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Haematological alterations in naturally occurring *Babesia vogeli* infections in dogs#

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The present study was conducted in 200 client-owned dogs presented to the University veterinary hospitals, Kokkalai and Mannuthy during the period from January 2023 to December 2023, with clinical signs of inappetence, pyrexia, pale mucous membrane and lethargy suggestive of babesiosis. Among the 200 dogs screened using PCR targeting the 18S rRNA gene of B. vogeli, a higher percentage of positive cases (31.5%) was found compared to light microscopy (25%), and these dogs underwent haematological analysis. The severity of the disease was assessed based on the variations in haematological parameters which were compared with values of 60 healthy dogs. Haematological analysis of affected dogs revealed mild anaemia and moderate thrombocytopaenia with highly significant variation in total lymphocyte count, total erythrocyte count, haemoglobin concentration, the volume of packed red cells, mean corpuscular haemoglobin concentration, platelet count and plateletcrit in infected animals compared to healthier ones. The findings of the current study indicated that all the biomarkers of anaemia and thrombocytopaenia could be used as a prognostic indicator in canine babesiosis caused by Babesia vogeli in field conditions and veterinary facilities.

Keywords: Dogs, anaemia, *Babesia vogeli*, thrombocytopenia, plateletcrit

Babesiosis in dogs could be caused by various species including *Babesia gibsoni* (*B. gibsoni*), *Babesia vogeli* (*B. vogeli*), *Babesia canis* (*B. canis*), *Babesia rossi* (*B. rossi*), *Babesia conradae* (*B. conradae*), *Babesia vulpes* (*B. vulpes*), and the newly described *Babesia negevi* (*B. negevi*) (Birkenheuer, 2023; Abdoli *et al.,* 2024; Malinovska, 2024). In India, canine babesiosis is caused by two main species, *B. vogeli* and *B. gibsoni*, both globally distributed and well-known among the domestic dog population, elucidating 'parasite globalization' (Mittal *et al.,* 2019; Anju *et al.,* 2022). Babesiosis is prevalent in south India as one of the most common tick-borne parasitic diseases of dogs, attributed to either the small piroplasm (*B. gibsoni*) or the large piroplasm (*B. vogeli)* (Jain *et al.,* 2017; Arun *et al*., 2022).

Babesia vogeli is a large piroplasm, about twice the size of *B. gibsoni*, with a pyriform shape, often found in pairs, and transmitted by *Rhipicephalus sanguineus sensu lato* ticks. It is primarily prevalent in tropical and subtropical regions

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(Boozer and Macintire, 2003; Solano-Gallego and Baneth, 2011). Haematology plays a crucial role in diagnosing large babesia species, as the presence of anaemia and thrombocytopaenia, along with a relevant clinical history, can indicate active babesiosis. The differential white blood cell counts are an easily measurable, available and reliable parameter which can be used as a severity index of canine babesiosis (Kucer *et al.,* 2008). As the clinical signs of babesiosis are not always very specific, a warning system based on routine haematology bloodwork would offer advantages (Pijnacker *et al.,* 2022).

Data on haematological alterations in dogs suffering from clinical babesiosis remains limited and incomplete. The most frequently reported haematological findings in canine babesiosis were mild to moderate anaemia (mean packed cell volume (PCV) of 26.4%), thrombocytopenia (mean platelets of 204.1×10^6/µL) and leukopenia with neutropenia and/or lymphopenia (Lee *et al.,* 2009; Scheepers *et al.,* 2011; Solano-Gallego *et al.,* 2016). Hence the current study was conducted to ascertain the haematological variations due to naturally occurring *B. vogeli* infections in dogs of Thrissur, Kerala.

Materials and methods

Two hundred client-owned dogs presented to the University veterinary hospitals, Kokkalai and Mannuthy with clinical signs of inappetence, pyrexia, anaemia, pale mucous membrane, haemoglobinuria, generalised lymphadenopathy, lethargy and weakness suggestive of babesiosis were included in the study. Dogs of any breed, age and sex were included. Peripheral blood smears of these animals were examined using Field's staining technique. The molecular screening for *B. vogeli* was conducted as per Duarte *et al.* (2008) with the forward primer BAB1 (5'-GTG AAC CTT ATC ACT TAA AGG-3') and reverse primer BAB4 (5'-CAA CTC CTC CAC GCA ATC G-3'), targeting the *18S rRNA* gene of the protozoan. The amplification was performed in an automated thermal cycler (BIORAD C1000 Touch™, Poland) with the following cycling conditions: initial denaturation at 94˚C for 2 min, 35 cycles of 94˚C for 30 seconds, 57.9˚C for 30 seconds, 72˚C for 30 seconds and final extension at 72˚C for 5 minutes (Ajith *et al.,* 2024).

The haematological analysis was performed in *B. vogeli* PCR-positive animals. Two millilitres of blood were aseptically drawn from either the medial cephalic vein or the saphenous vein of each dog and placed into an EDTA-coated tube. Complete blood count including the parameters haemoglobin (g/dL), volume of packed red cells (%), total erythrocyte count (TEC) (10^6/mm3), total leukocyte count (TLC) (10^3/mm3), differential leukocyte count (per cent), mean corpuscular volume (MCV) (fL), mean corpuscular haemoglobin (MCH) (pg), mean corpuscular haemoglobin concentration (MCHC) (g/dL), thrombocyte count (10^3/µL), mean platelet volume (MPV) (fL), plateletcrit (PCT) (%) and platelet distribution width (PDW) (fL) was done using automatic haematological analyser (Mythic 18 Vet, Poland). The data were expressed as the mean ± standard error. The parameters of affected animals were compared with those of healthy dogs (n = 60) that visited the hospital for vaccination and routine health check-ups over the same period.

The severity of anaemia and thrombocytopenia were classified as per Ionita *et al.* (2023). The severity of anaemia (lower values for the PCV and/or haemoglobin (Hgb)) was defined as follows: mild (30% ≤ PCV < 37% and/or 10 ≤ Hgb < 12 g/dL), moderate (20% ≤ PCV < 30% and/or 5 ≤ Hgb < 10 g/dL), or severe (PCV < 20% and/or Hgb < 5 g/dL). The severity of thrombocytopenia (lower values for the platelet count (PLT)) was defined as follows: mild ($100 \le PLT < 200\times10^9$/L), moderate ($50 \le PLT < 100 \times 10^9$/L), or severe ($PLT < 50 \times10^9$/L).

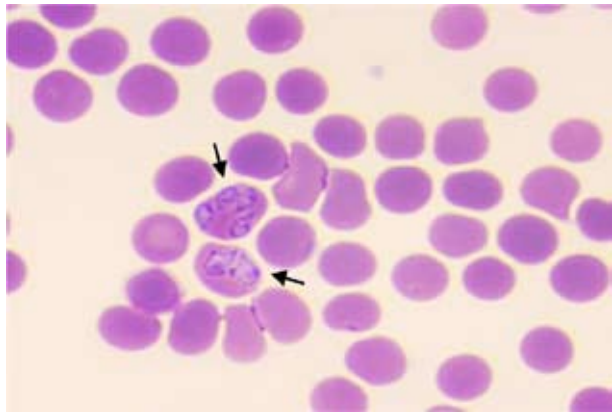

Fig. 1. Intraerythrocytic merozoite of *B. vogeli* in the Field's-stained smear (×1000) showing paired or tear shaped pyriform bodies with basophilic cytoplasm and reddish chromatin (Black arrows).

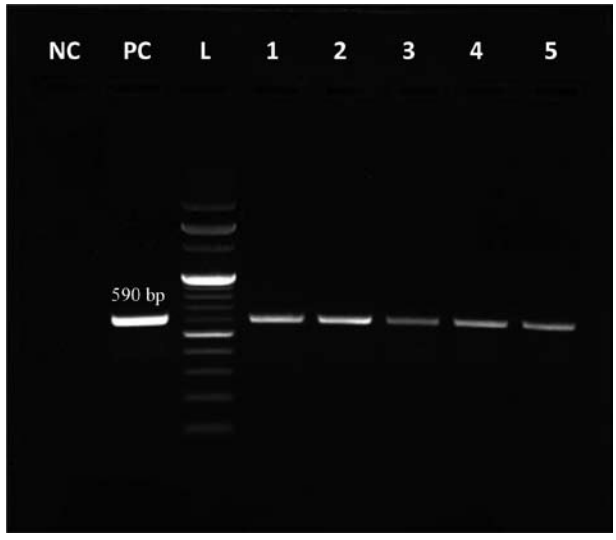

Fig. 2. Agarose gel electrophoresis of *18S rRNA* gene of *B. vogeli.* Lane L: 100 bp ladder, Lane NC: negative control, Lane PC: positive control, Lane 1,2,3,4,5: positive samples.

According to the central limit theorem, our sample was sufficiently large (N>30) which revealed a normal distribution for the use of parametric tests (Liu *et al.,* 2022). Haematological parameters were evaluated statistically using the statistical package for social sciences (SPSS Version 24.0), comparing means using independent samples T test.

Results and discussion

The present study included 200 dogs of various breeds and age groups presented from January 2023 to December 2023 with different clinical conditions at the outpatient unit of university veterinary hospitals, Kokkalai and Mannuthy. The age of the study population varied from seventy-five days to eighteen years and included both male and female animals of different breeds, including mixed breeds, from both the diseased and healthy groups. Microscopic examination of the Field's-stained peripheral blood smears from dogs with clinical signs of fever, lymphadenopathy, anorexia etc. revealed intra-erythrocytic piroplasm of *B. vogeli* organisms which were pear-shaped and in pairs among 50 (25 per cent) dogs (Fig.1). Out of the 200 animals screened by PCR, 63 (31.5 per cent) animals yielded a specific PCR product of approximately 590 base pair with primer pair BAB1 and BAB4 (Fig. 2). The BLAST analysis confirmed 99.23 per cent similarity to the sequences of *B. vogeli* with that available in GenBank database (Thrissur-MN190278, Texas-EU084675, New Delhi-MN165667 and Gujarat-MZ646048).

Babesiosis is a globally recognized tick-borne disease in dogs causing anaemia (Liu *et al.,* 2022). It could be caused by various species including *B. canis, B. rossi, B. vogeli, B. gibsoni, B. conradae, B. vulpes* and the newly described *B. negevi* (Birkenheuer, 2023). The presence of *B. canis* was first described in the year 1895 by Piana and Galli-Valerio in a hunting dog in Italy (Troskie *et al.,* 2019). Currently, *B. vogeli, B. canis* and *B. rossi* are categorized as the large species of canine babesia (Panti-May and Rodiguez-Vivas, 2020). This large babesia parasites of dogs were transmissible by ticks of the genera *Dermacentor, Haemaphysalis* and *Rhipicephalus* (Uilenberg, 2006).

The haematological findings in blood samples of *B. vogeli-infected* dogs by PCR (n=63) were compared with healthy animals (n=60), and are shown in Table 1. Highly significant difference in means of total lymphocyte count (3.56 ± 0.55×10^3/µL), total erythrocyte count (4.63 ± 0.21×10^6/µL), haemoglobin concentration (11.40 ± 0.53 g/dL), volume of packed red cells (31.03 ± 1.40%), mean corpuscular haemoglobin concentration (36.45 ± 0.44 g/dL), platelet count (84.14 ± 8.67 ×10^3/µL) and plateletcrit (0.08 ± 0.03%) were observed in infected animals compared to 60 healthier ones. However, when compared to normal reference values, the total lymphocyte counts and MCHC values were in the normal range. There was a significant reduction in the total erythrocyte count, haemoglobin concentration, volume of packed red cells, mean corpuscular haemoglobin concentration, platelet count and plateletcrit.

Anaemia is a decrease in red blood cells (RBC), volume of packed red cells (VPRC), and haemoglobin in the blood due to haemolysis, haemorrhage, or decreased production of red blood cells. Severity, RBC indices, and regenerative response generally characterise anaemia (Abakpa *et al.,* 2023). Anaemia is a clinical and laboratory sign, not a disease resulting from different primary conditions and diseases (Yadav *et al.,* 2022). In the current study, the prevalence of anaemia in dogs with canine babesiosis was 63.5 per cent, which is higher than the 44.83 per cent reported by Bhat *et al.* (2016), but consistent with the results of Abakpa *et al.* (2023). According to Fabisiak *et al.* (2010), anaemia has been reported as the hallmark of canine babesiosis resulting from intravascular and extravascular haemolysis. The high prevalence of anaemia, mostly mild, recorded in this study corroborates the report of the same researcher.

The most consistent features observed by Preena *et al.* (2021) in large babesia-infected dogs of Kerala (Kannur district) were normocytic, mild regenerative and normochromic anaemia and moderate thrombocytopenia. *Babesia* spp. are believed to induce anaemia through the destruction of red blood cells mediated by antibodies and the direct damage inflicted on these cells by the parasites themselves causing both extravascular and intravascular haemolysis. The decrease in volume of packed red cells (31.03 ± 1.40 %) found in this study was hypothesised to be caused by haemodilution, splenomegaly and sequestration (Scheepers *et al.*, 2011). Extravascular haemolysis occurs due to the activity of phagocytes in the spleen and liver, while intravascular haemolysis is driven by both the parasite's lifecycle (specifically merogony) and immune-mediated destruction of red blood cells (Zygner *et al.,* 2023). Sangeetha and Raguvaran (2022) noticed regenerative immune-mediated haemolytic anaemia, non-regenerative anaemia, leucocytosis, leucopenia, thrombocytopenia, febrile illness and splenomegaly as the main clinicopathological findings in *B. vogeli* infections of dogs.

In canine babesiosis, the primary cause of anaemia is not the direct destruction of infected red blood cells by the parasite. The lack of association between the levels of parasitaemia and anaemia in Babesia-infected dogs in general, indicated that immune responses contribute to decreasing the RBC count. During both malaria and babesiosis, the level of parasitaemia does not correlate with the severity of anaemia (Kelly *et al.,* 2015). Decreased haematocrit is a prognostic marker in large babesia-infected dogs and is significantly lower in non-survivors in comparison to survivors (Eichenberger *et al.,* 2016). In acidic and hypercapnic conditions, *B. vogeli*

Table 1. Haematological parameters recorded in *B. vogeli* infected dogs (n = 63) and healthy dogs (n = 60)

Haematological parameters	Reference levels	Mean ± SE		t-value	p-value
		Infected group	Healthy group		
Total leucocyte count (×10³/µL)	6.0 - 17.0	11.97^{a} ± 1.12	11.00^{a} ± 0.49	0.79^{ns}	0.433
Total lymphocyte count (×10³/µL)	0.7 - 5.1	3.56^{a} ± 0.55	2.20^{b} ± 0.16	2.36	0.022
Total monocyte count (×10³/µL)	0.2 - 1.7	0.94^{a} ± 0.13	0.89^{a} ± 0.06	0.34^{ns}	0.736
Total granulocyte count (×10³/µL)	4.4 - 12.6	7.50^{a} ± 0.70	7.86^{a} ± 0.37	-0.46^{ns}	0.648
Lymphocyte (%)	12.0 - 30.0	27.02^{a} ± 1.92	20.10^{b} ± 0.95	3.21	0.002
Monocyte (%)	3.0 - 10.0	7.68^{a} ± 0.58	7.10^{a} ± 0.44	0.79^{ns}	0.429
Granulocyte (%)	60.0 - 74.0	65.30^{b} ± 1.20	72.27^{a} ± 0.88	-3.19	0.002
Total erythrocyte count (×10⁶/µL)	5.50 - 8.50	4.63^{b} ± 0.21	6.16^{a} ± 0.10	-6.59	<0.001
Haemoglobin (g/dL)	12.0 - 18.0	11.40^{b} ± 0.53	14.45^{a} ± 0.20	-5.36	<0.001
Volume of packed red cells (VPRC) (%)	37.0 - 55.0	31.03^{b} ± 1.40	42.97^{a} ± 0.58	-7.92	<0.001
Mean cell volume (MCV) (fL)	60.0 - 77.0	67.51^{a} ± 1.04	69.66^{a} ± 0.73	-1.69^{ns}	0.093
Mean corpuscular haemoglobin (MCH) (pg)	19.0 - 25.0	24.56^{a} ± 0.43	24.01^{a} ± 0.31	1.04^{ns}	0.303
Mean corpuscular haemoglobin concentration (MCHC) (g/dL)	32.0 - 36.0	36.45^{a} ± 0.44	34.24^{b} ± 0.37	3.83	<0.001
Platelet count (×10³/µL)	160.0 - 525.0	84.14^{b} ± 8.67	317.82^{a} ± 9.86	-17.79	<0.001
Mean platelet volume (MPV) (fL)	7.0 - 13.0	9.40^{a} ± 0.30	9.93^{a} ± 0.32	-1.21^{ns}	0.229
Plateletcrit (PCT) (%)	0.15 - 0.39	0.08^{b} ± 0.03	0.25^{a} ± 0.01	-4.56	<0.001
Platelet distribution width (PDW) (fL)	51.0 - 73.0	14.34^{a} ± 0.64	14.89^{a} ± 0.72	-0.57^{ns}	0.568

Different superscripts indicate mean values differ significantly at 0.05 level (p<0.05). Superscript 'ns' indicates mean value does not differ significantly at 0.05 level (p<0.05)

causes dysfunction of remaining haemoglobin in intact cells by producing enzymes that cleave haemoglobin, as well as a qualitative and quantitative deficit of haemoglobin (Omobowale *et al.,* 2017).

Phagocytosis, oxidative damage to red blood cells, antibodies, and the complement system all play roles in responding to infection. These mechanisms contribute to the elimination of the parasite and the development of anaemia, with more severe cases potentially leading to additional complications. Moreover, besides immune-mediated haemolytic anaemia, sequestration of erythrocytes in microvasculature and splenic retention of RBCs may also negatively impact the RBC count in infected dogs (Zygner *et al.,* 2023). Although low levels of parasitaemia along with severe anaemia can result from the sequestration of infected red blood cells in micro-vessels, the immune response still plays a crucial role in both eliminating the parasite and destroying red blood cells (Schetters, 2019).

Thrombocytopaenia, a low platelet count was frequently encountered and considered to be the hallmark of babesiosis (Coralic *et al.,* 2018). The MPV generally increased, suggesting there was a normal bone marrow response to the use, sequestration, or destruction of platelets. Possible mechanisms of thrombocytopenia included local and systemic disseminated intravascular coagulation (DIC), immune-mediated destruction, and sequestration of platelets in the spleen (Macintire *et al.,* 2002; Boozer and Macintire, 2003). According to Yousaf *et al.* (2024), thrombocytopaenia in babesiosis was caused by immune mechanisms, splenic sequestration, or coagulatory consumption of platelets from haemolytic or vascular injury.

Modern cell analysers routinely investigate platelet indices, such as MPV, PCT and PDW, that could be utilized in diagnosis and decision-making in babesiosis. Zvorc *et al.* (2010) defined PCT as the percent of blood volume occupied by platelets. In this study, platelet number and plateletcrit were significantly decreased, whereas the MPV and PDW were in the normal range. Plateletcrit is an indicator of platelet mass in the body, whereas MPV represents a marker of platelet function and activation (Baranidharan *et al.,* 2017). Goddard *et al.* (2015) reviewed platelet as well as platelet indices as surrogate markers of platelet activation associated with disease outcome in canine babesiosis. Plateletcrit in blood samples represents the most relevant physiological parameter of platelet status and acts as an indicator of platelet mass in the body, just as haematocrit is an indicator of total erythrocyte mass in the body (Bommer *et al.,* 2008).

Conclusion

The present study assessed haematological changes in dogs naturally infected with *B. vogeli* by utilizing PCR targeting the *18S rRNA* gene of the pathogen. Among the 200 dogs examined, PCR revealed a higher positivity rate of 31.5 per cent, while light microscopy detected only 25 per cent. Most dogs with naturally occurring *B. vogeli* infections exhibited mild anaemia (characterized by normal or slightly reduced haemoglobin levels and packed cell volume) and moderate thrombocytopenia with decreased plateletcrit values. The level of parasitaemia in *B. vogeli* infections was found to be very low, making it likely that such infections could be missed during routine blood smear examinations. Additionally, natural blood parasite infections occurred as either mono-infections or co-infections. Therefore, it is advisable to complement blood smear results with other confirmatory or advanced molecular tests to detect potential co-infections with other tick-borne pathogens in more severe cases.

Conflicts of interest

There are no conflicts of interest reported by the authors.

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