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Evaluation of blood parameters in naturally infected dogs with *Babesia gibsoni* and *B. vogeli*

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Abstract
Canine babesiosis is an emerging disease with worldwide distribution. There are few studies about the haematological and biochemical findings of canine babesiosis. Therefore, the present study was conducted to understand the hematobiochemical changes in PCR confirmed *Babesia gibsoni* (n = 21) and *B. vogeli* (n = 22) blood samples from dogs. It was found that the *B. gibsoni* and *B. vogeli* infected dogs have significant reduction in Hb, PCV, TEC and TLC values. However, there was no significant changes in liver (ALT) and kidney (creatinine) function tests compare to uninfected healthy dogs. Thrombocytopenia, on the other hand, was statistically recognised only in dogs infected with *B. gibsoni*. From the present study, it was found that the anaemia and thrombocytopenia may server as early indicators for clinical diagnosis of babesiosis for initiating treatment before confirmatory diagnosis with molecular assays such as PCR in field conditions.

Keywords: Anaemia, *B. gibsoni, B. vogeli*, dogs, thrombocytopenia

Introduction
Babesiosis is one of the most important and common vector-borne infection of dogs, and in India it is primarily caused by *B. gibsoni* and *B. vogeli.* The infections are mainly transmitted by *Rhipicephalus sanguineus* and *Haemaphysalis longicornis* ticks (Shaw *et al*., 2001) [15]. However, other routes such as dog bites, blood transfusions, and the transplacental route are involved in the transmission (Fukumoto *et al*., 2005, and Jefferies *et al*., 2007) [4, 9]. The acute form of the disease is typically associated with fever, lethargy, pale mucus membranes, lymph node enlargement, vomiting, diarrhoea, melena, dark yellow urine, marked splenomegaly, and hepatomegaly (Boozer and Macintire, 2003) [3], and the chronic form of infection, which is frequently encountered is very difficult to diagnose. Among the *B. gibsoni* and *B. vogeli* infections, *B. gibsoni* generally result in severe clinical manifestations (Harikrishnan *et al*., 2002) [6]. There are very few studies from Karnataka with respect to haemato-biochemical abnormalities in *Babesia* sp. infected dogs. As a result, the current study was designed to assess detailed haematological and biochemical changes in both *B. gibsoni* and *B. vogeli* infections in dogs.

Material and Methods
The blood samples from dogs were collected with the owners' consent from pet practitioners in and around Bengaluru. A total of 328 blood samples were collected from dogs and among them a total of 21 from *B. gibsoni* and 22 from *B. vogeli* infected (confirmed by PCR) and 14 uninfected blood samples were included for the study. All the blood samples were subjected to microscopy for detection of haemoprotozoa organisms and individual PCRs for confirmation of *B. gibsoni* and *B. vogeli* and were also tested negative for *Hepatozoon canis*, and *Ehrlichia canis.*

The blood samples were processed to record the parameters, viz., total erythrocyte count (TEC) (10^6/µl), total leucocyte count (TLC) (10^3/µl), haemoglobin (Hb) (g %), packed cell volume (PCV) (%) and platelet count (10^3/µl) with an automated cell counter (Mindray, BC 2800). The serum samples were subjected for alanine transaminase (ALT) (U/dl) and creatinine (mg/dl) estimation by semi-automatic biochemical analyzer (Mindray, BS-120) using commercial kits. The statistical analysis to compare the mean values between the control and infected groups was carried out by Pearson's chi-square ($\chi 2$) test using the Graphpad Prism5 statistical software programme as described by Snedecor and Cochran (1994) [16].

Results

A total of 43 blood samples were confirmed positive for *Babesia* sp. by microscopy (Fig. 1a and 1b) and PCR for individual detection of *B. gibsoni*, *B. vogeli* (Lavanya *et al*., 2019a & 2019b) [10, 11], *Hepatozoon canis* (18s rRNA) and *Ehrlichia canis* (16s rRNA) organisms. Out of that, 21 blood samples were confirmed for *B. gibsoni* and 22 were for *B. vogeli*. For control uninfected blood samples of 14 healthy dogs aged less than 2 years were subjected to PCR to rule out all four protozoa infections by PCR.

The haematological analyses revealed, a significant ($p<0.01$) decrease in the haemogram viz. Hb, TEC and PCV and platelet count and a significant decrease in TLC ($p=0.042$) in *B. gibsoni* infected dogs (n=21) compared to the healthy dogs (n=14). In biochemical analysis, there was no statistically significant changes in the values of ALT ($p=0.06$) and creatinine ($p=0.62$) (Table 1).

The haemato-biochemical analyses revealed, a significant decrease ($p<0.01$) in the Hb, TEC and PCV and TLC ($p=0.038$) in *B. vogeli* infected dogs (n=22) compared to the healthy dogs (n=14). There was no statistically significant change in the values of platelet counts ($p=0.169$), ALT ($p=0.658$) and creatinine ($p=0.15$) (Table 1).

Discussion and Conclusion

Canine babesiosis is a growing concern for clinicians due to the overlapping of clinical signs and lab findings exhibited, leading to an elusive diagnosis. The disease causes high mortality in puppies, and infection persists in aged animals both in urban and rural areas (Irwin, 2009) [7]. Therefore, the present study was undertaken to evaluate the blood parameters of 43 dog samples that were confirmed positive for *Babesia gibsoni* (n = 21) and *B. vogeli* (n=22) both by microscopy and individual PCRs, and to know the clinical indicator for early diagnosis before confirming the results any means of other sophisticated technique.

The haemato-biochemical values of both *B. gibsoni* and *B. vogeli* infected dogs, showed statistically significant reduction in Hb, PCV, TLC and TEC values and non-significant values were observed in ALT and creatinine. The statistically significant reduction in Hb, PCV, TLC and TEC values in *B. gibsoni* infected dogs were in agreement with Sundar *et al*. (2004) [17], Bilwal *et al*. (2017) [2], Gonde *et al*. (2017) [5] and Jain *et al*. (2017) [8] who also observed anaemia and thrombocytopenia. It is theorised that the anaemia in babesiosis results from increased osmotic fragility of erythrocytes, increased erythrophagocytic activity of macrophages, and immune-mediated cleavage (Makinde and Bobade, 1994 and Murase *et al*., 1996) [12, 13]. Significant thrombocytopenia could be due to platelet sequestration in the spleen or immune mediated destruction and development of disseminated intravascular coagulation (Boozer and Macintire, 2003) [3]. The non-significant ALT and creatinine values of *B. gibsoni* infected dogs were in agreement with Sundar *et al*. (2004) [17], who also recorded no alterations in ALT and creatinine levels. In contrast, Bilwal *et al*. (2017) [2] and Gonde et al. (2017) [5] recorded significantly increase in ALT levels in infected dogs.

The haemato-biochemical values of *B.vogeli* were in agreement with Reddy et al. (2014) [14], Gonde et al. (2017) [5] and Bai *et al*. (2019) [1], who also revealed significant reduction in Hb, TEC, TLC and PCV values, but contrastingly, they found significant reductions in platelet counts and increase in ALT and creatinine. In the present study, the normal values of platelets, creatitine, and ALT in *B. vogeli* infection in dogs may be due to a long standing infection attributable to the dogs' good immune status. Thus, it can be concluded that, dogs showing laboratory findings of decreased Hb. TEC, PCV and platelet counts should be suspected for *B. gibsoni* infection and only reduction in Hb. TEC, and PCV values should be suspected for *B. vogeli* infection in dogs along with clinical symptoms for initiating early treatment before awaiting for results from other advanced techniques such as PCR, which may not possible in all the clinical cases that to in rural and peri urban setup where the availability of diagnostic facilities in minimal or many times there no such facilities .

Table 1: Haemato-biochemical alterations in dogs infected with *B. gibsoni* and *B. vogeli*

Parameter	Healthy (n=14)	*B. gibsoni* (n=21)			*B. vogeli* (n=22)		
		Mean ± SE	t-test	p value	Mean ± SE	t-test	p value
TEC ($x10^6$/µl)	7.10 ±0.18	2.90±0.25	11.89	< 0.0001**	4.49±0.43	4.512	0.0001**
Hb (g/dl)	15.78±0.47	6.63±0.57	11.32	<0.0001**	9.19±0.99	5.049	<0.0001**
PCV (%)	46.95±1.56	20.03±1.68	11.09	<0.0001**	27.04±2.84	5.245	<0.0001**
TLC ($x10^3$/µl)	15.41±1.06	22.49±2.62	-2.12	=0.0420*	27.97±4.57	2.156	0.0382*
Platelets ($x10^3$/µl)	274.92±31.8	78.14±25.15	4.88	<0.0001**	193.54±41.4	1.404	0.1693NS
ALT (units/L)	26.42±2.82	19.74±2.09	1.93	0.0612NS	24.49±2.93	0.446	0.6581NS
Creatinine (mg/dl)	0.87±0.04	0.78±0.15	0.50	0.6250NS	1.39±0.28	-1.448	0.1517NS

*Significant ($p<0.05$)
** Highly significant ($p<0.01$)
NS: Non significant ($p>0.05$)

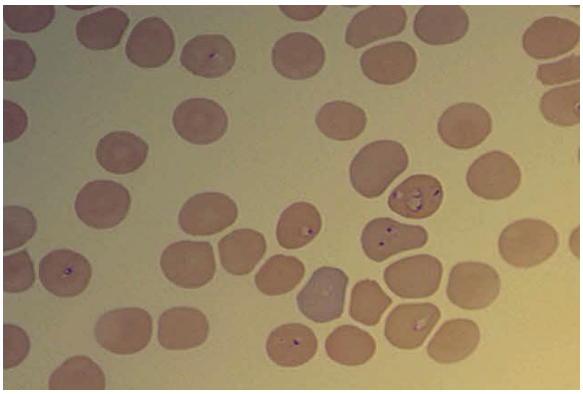

Fig 1a: *B. gibsoni* in giemsa-stained blood smear (1000x) showing signet ring and multiple organisms within erythrocytes

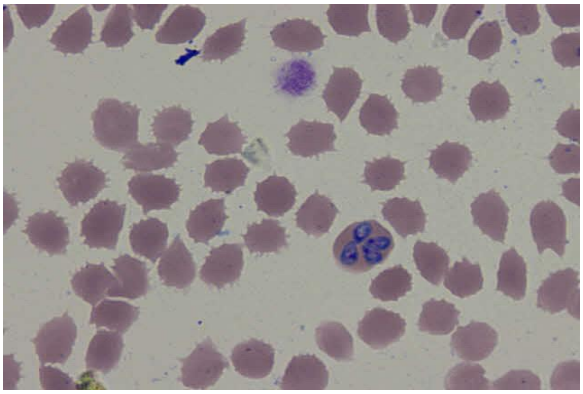

Fig 1b: *B. vogeli* in giemsa-stained blood smear (1000x) showing pear shaped and multiple organisms within erythrocyte.

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