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PREVALENCE AND HAEMATOLOGICAL ALTERATIONS ASSOCIATED WITH *Babesia gibsoni* INFECTION IN CANINE POPULATION OF KANNUR DISTRICT, KERALA

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ABSTRACT

A total of 2,345 dogs suspected for babesiosis presented to District Veterinary Centre, Kannur were screened for detection of intraerythrocytic piroplasm of *Babesia* species by light microscopic examination of Giemsa stained blood smears, during the period from November 2018 to December 2019. Haematology was performed using automated analyzer to ascertain the impact of the disease in affected animals. Statistical analysis of haematological parameters of different groups based on degree of parasitaemia was also performed. This communication places on record – an overall prevalence of 14.5% babesiosis with a predominance of *Babesia gibsoni* infection (13.9%) with two notable seasonal peaks (pre-monsoon and post-monsoon). Most significant haematological abnormality associated with *B. gibsoni* infection was anaemia and thrombocytopenia. In addition, neutropenia and lymphocytosis were also recorded in affected dogs. The typical blood picture findings revealed variable degree of regenerative anaemia with polychromasia, macrocytes, anisocytosis, nucleated RBCs (metarubricytes & rubricytes), Howell-Jolly bodies and moderate to severe thrombo cytopenia along with macroplatelets and platelet aggregation. Besides, a low prevalence of *B. canis* infections (0.60%) was also noted in the study.

Keywords: *Babesia gibsoni, B.canis*, Dogs, Kerala, Prevalence

INTRODUCTION

Canine babesiosisis is one of the commonly presented tick-borne diseases caused by intraerythrocytic protozoa of the genus *Babesia,* classified under the phylum Apicomplexa. In the absence of suitable tick vector (*Rhipicephalus sanguineus, Haemaphysalis longicornis, H. bispinosa* etc.), direct transmission between dogs presumably by fighting, physical contact, transplacental transmission, therapeutic blood transfusion and needle passage during experimental infections may occur (Jefferies *et al*., 2007). The disease exhibits a wide range of presentations from subclinical infection to extensive organ damage, even turning fatal, at times. The clinical manifestations are characterized by fever,

splenomegaly, weakness, multi-organ failure and collapse associated with intravascular and extravascular haemolysis whereas subclinical or chronic infections are often asymptomatic. Two species of Babesia namely *B.canis vogeli* (large form) and *B.gibsoni* (small form) are reported from India with significant prevalence of the latter (Jain *et al*., 2017). *Babesia gibsoni* is a small, pleomorphic, oval to annular shaped parasite with less than 3µm length, occupying less than one-eighth of the diameter of the host erythrocyte (Vishnurahav *et al*., 2014). It was first reported in hounds and jackals by Patton in India (Patton, 1910). Microscopic examination of the stained peripheral blood smears were used for preliminary diagnosis of the condition. However, low parasitaemia in case of subclinical or chronic cases may not be detectable by this method. Even then, it continues to be the quickest confirmatory method for large scale screening of diseases and hence was followed in the present study. The present longitudinal study is to find the prevalence of *Babesia spp.* infection in dogs using conventional microscopy and to ascertain its haematological alterations with respect to the grades of parasitaemia.

MATERIALS AND METHODS

a. Study population

The present study was conducted in 2,345 dogs of various breeds and age groups from different parts of Kannur district enrolled in outpatient ward, District Veterinary Centre, Kannur (Kerala) during the period from November, 2018 to December, 2019, with clinical signs like anorexia, lymphadenopathy, tick infestation, weakness, pallor of mucous membranes, fever and jaundice suggestive of babesiosis.

Light microscopic examination

Peripheral blood was collected from ear tip, thin smear prepared on a clean glass slide, fixed in absolute methanol and stained using Giemsa stain. The slides were screened under 1000X for the presence of piroplasms of the *Babesia* spp. in erythrocytes.

b. Haematological examination

Around 2 ml of blood was collected from either the cephalic vein or recurrent tarsal vein in EDTA vials. Haematological analysis was performed on an automatic haematology analyzer (Exigo EOS, Sweden). Haemoglobin (Hb), total leucocytes, differential leucocyte count and platelet count were ascertained.

c. Statistical analysis

The animals were grouped based on the degree of parasitaemia of the most prevalent species (Table 1) as described by Vinodkumar *et al.* (2016). The data obtained were represented as mean ± standard deviation. Haematological parameters were evaluated statistically using the Statistical Package for Social Sciences (SPSS Version 20.0.0), comparing means using one-way ANOVA with Duncan's multiple range test.

Table 1. Classification of infection based on the level of parasitaemia

P1	Stray organisms detected (<1% of erythrocytes affected).
P2	1 organism detected in most of the fields (5% of erythrocytes affected).
P3	2-3 organisms detected in most of the fields (<10% of erythrocytes affected)
P4	4 or more organisms detected in most of the fields (>10% of erythrocytes affected)

RESULTS AND DISCUSSION

In the present study, intra-erythrocytic piroplasms of *Babesia* were detected by microscopy in 340 (14.5%) out of a total of 2,345 samples examined. Species wise predominance of *Babesia gibsoni* (13.9%) was found in comparison to *B. canis* (0.6%) in the study population. Diagnosis of babesiosis in animals is usually done by examination of piroplasms in Giemsa stained peripheral blood smears. Besides, acridine orange staining technique was found to be accurate, simple, sensitive and rapid when compared to Giemsa staining for screening of haemoprotozoans and rickettsiales in animals (Ravindran *et al.,* 2007). Low parasitaemia, subclinical and chronic infections demand advanced techniques of diagnosis with greater sensitivity, like percoll gradient peripheral centrifugation, molecular techniques (PCR), serologic testing (IFAT or ELISA) and flow cytometry (De Gopegui *et al.,* 2007). The prevalence pattern of the two *Babesia* species observed in the present study was found similar to the records of other authors from various parts of India (Varshney *et al.,* 2003; Laha *et al.,* 2014; Augustine *et al.,* 2017; Jain *et al*., 2017).

B.gibsoni was found to be the most prevalent haemoparasite in southern and north-eastern India, but rare in other parts of northern and central India (Sarma *et al.,* 2019). It was also reported that canine babesiosis had an endemic status in South India with significantly higher proportion of *B.gibsoni* in both suspected and healthy animals indicating the carrier status of the pathogen (Jain *et al*., 2017). After the first report of *B.gibsoni* in Kerala by Sabu (2005), the decade has witnessed a sharp increase in the prevalence of the infection. This increased prevalence may be due to complex interaction with factors such as climate, habitat management practices, subclinical or chronic status of infection, increased vector population and distribution (Jain *et al*., 2017).

The incidence of *B.gibsoni* was observed throughout the study period; however, it showed two peaks of occurrence (Fig.1), a higher peak in post monsoon (October-February, 2018) and another peak in monsoon (June-July, 2019). Similar starting of a new peak could be noted in the next post monsoon cycle from the month of November 2019; however, the fate of peak is

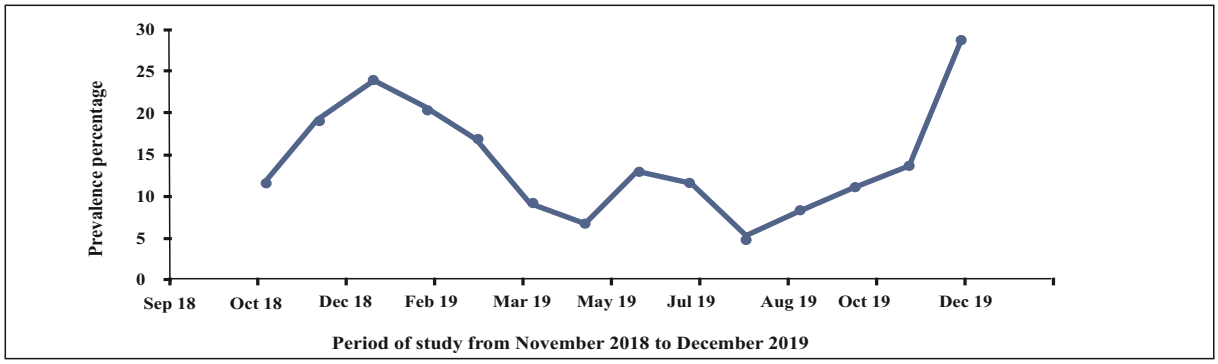

Fig. 1: Monthly prevalence of *B. gibsoni* infection during the study period

undetermined as it was not involved in the period of study. Similarly, two peaks of occurrence of canine babesiosis in the two seasons (summer and winter) was reported by Kumar *et al.* (2009). On the contrary, it was recorded that risk factors like age, sex, breed and season had no significance in the occurrence of canine babesiosis (Augustine and Sabu, 2014).

On examination of blood smears, *B. gibsoni* infected cases were classified based on the grade of parasitaemia as P1-P4. Table 2 summarizes the haematological parameters of different classes (P1-P4) and healthy class (P5). Fig. 2-4 depicts blood picture of *B. gibsoni* infected RBCs. Anaemia of variable severity was observed in all the infected dogs as similarly reported by Yogesh priya *et al.* (2018). There was significant difference in the level of Hb in different classes of infection and that of normal animals ($p<0.05$). In the infected animals, Hb level in P1 was significantly different from that of P2, P3 and P4 which might be due to mild degree of infection leading to low parasitaemia. However, the anaemic changes in P2, P3 and P4, though significantly lower than control group, did not differ significantly between each other despite the detectable difference in the intensity of parasitaemia, which was in correlation with the observations of Brown *et al*. (2015). He opined that the severity of anaemia was seldom proportional to the magnitude of the peripheral parasitaemia. The different grades of anaemia might be due to direct mechanical disruption of infected erythrocytes, intravascular haemolysis, oxidative, immune-mediated and non-immune mediated destruction of red blood cells (Meinkoth *et al*., 2002). It was also reported that the organism initiates an antibody-mediated cytotoxic destruction of circulating erythrocytes leading to immune mediated haemolytic anaemia and overwhelming host immune response in non-anemic babesiosis (Boozer and Macintire, 2003). The blood picture of regenerative anaemia was noted in all infected animals with different degree of polychromasia, macrocytes, anisocytosis, Howell-Jolly bodies, nucleated RBCs (nRBC) like metarubricytes and rubricytes as described by Simoes *et al.* (2011).

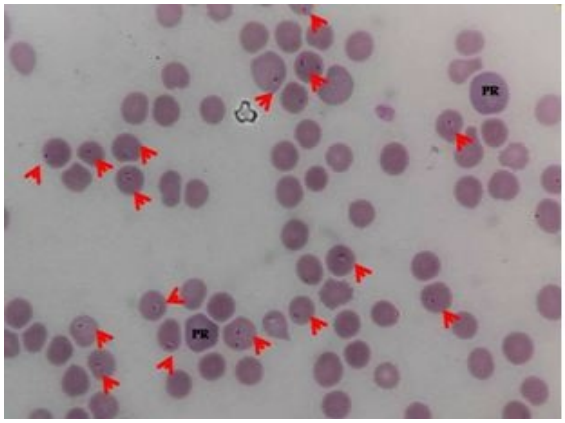

Fig. 2 More than 30% of RBCs could be found occupied by piroplasms of *B. gibsoni* (marked with red arrow head).

The level of parasitaemia was noted as P4 and few cells were polychromatophilic (PR) identified as immature anucleate erythrocytes with larger size and basophilic nature than normal RBC

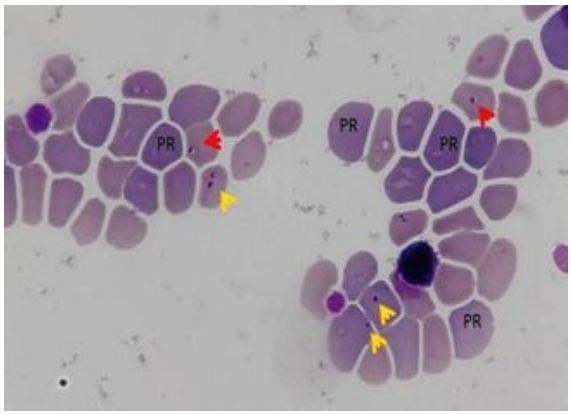

Fig. 3 Two RBCs (P3) could be found occupied by piroplasms of *B. gibsoni* (marked with red arrow head)

The whole blood picture indicated severe regenerative anaemia as shown by presence of numerous irregular shaped or macrocytic polychromatophils (labeled as PR), erythrocytic nuclear remnants or Howell Jolly bodies (marked with yellow arrow head)

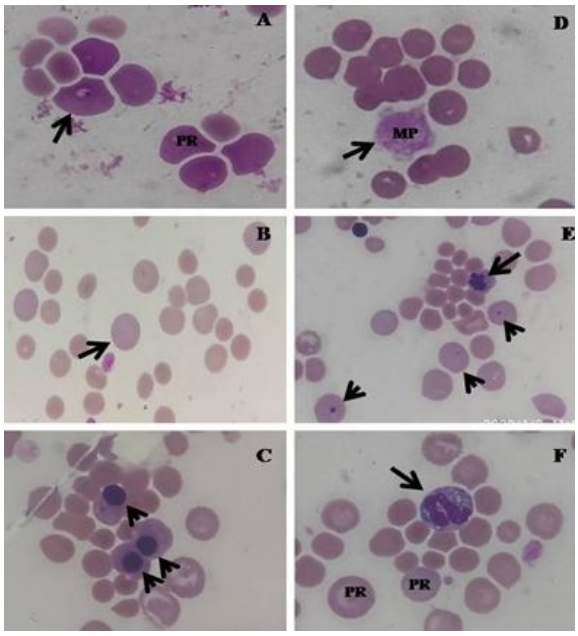

Fig. 4 A. *Polychromatophilic RBCs* were seen numerously, which are immature anucleated erythrocytes with bluish colour due to the presence of moderate to large amount of RNA (ribosomes, polyribosomes) which offsets the red of hemoglobin, imparting a purple colour to cells and a black arrow points the single piroplasm of *B. gibsoni*

B. *Macrocytic RBC* (black arrow) are larger cells which have normal content of hemoglobin and very little RNA and hence reddish in colour indicating a mounting regenerative response

C. *Polychromatophilic metarubricyte* are nRBCs with purple cytoplasm, pyknotic nucleus, irregular cytoplasmic edges (black arrow head)

D. *Macroplatelets* (MP) or giant platelets suggestive of regenerative response to thrombocytopenia (black arrow)

E. *Howell-Jolly bodies* (Blue round inclusion or micronuclei, black arrow head)

which are small fragments of non-functional nucleus which were not extruded as the erythrocyte left the bone marrow.

F. *Toxic neutrophils* observed which are segmented neutrophils with toxic change having less condensed chromatin, Dohle bodies, toxic cytoplasmic granulation and cytoplasmic basophilia & vacuolation.

There was no significant difference in total leucocyte count as reported in babesiosis by Jain *et al*. (2017). However, it was stated by Guitian *et al*. (2003) that leucogram in babesiosis may range between leucopenia to leukocytosis with the latter reaching extreme leukemoid counts in case of immune-mediated hemolytic anaemia. Moreover, neutropenia and lymphocytosis were noted in all the infected categories with a significant difference ($p<0.05$) from the normal animals. Toxic neutrophils exhibiting Dohle bodies, cytoplasmic basophilia and vacuolization, and reactive lymphocytes were observed indicating the bone marrow response to overwhelming infection. It could be suggested that the lymphocytosis noticed might be due to the prolonged or chronic infections with blood parasites. It could also be suggested that the nRBCs closely mimic the lymphocytes and the automated

Table 2: Haematological values of *Babesia gibsoni* infected dogs based on different level of parasitaemia (Mean ± Standard deviation)

Categories	P1	P2	P3	P4	P5
No. of animals	143	65	48	70	14
Hb (g/dl)	8.15±2.98^{a}	7.91±3.33^{b}	7.88±3.29^{b}	6.35±2.69^{b}	14.49±1.63^{c}
Total Count ($\times10^{3}$ /µl)	14.39±10.95^{NS}	13.64±7.95^{NS}	15.28±8.02^{NS}	15.73±13.38^{NS}	10.99±2.87^{NS}
Neutrophil (%)	57.38±12.33^{a}	59.83±16.28^{a}	60.65±11.13^{a}	59.43±10.96^{a}	71.57±6.37^{b}
Lymphocyte (%)	31.87±11.55^{a}	30.03±14.51^{a}	29.29±10.41^{a}	30.13±11.83^{a}	17.93±3.83^{b}
Monocyte (%)	9.65±4.98^{NS}	9.31±4.78^{NS}	8.65±3.65^{NS}	9.5±4.53^{NS}	8.5±2.79^{NS}
Platelet ($\times10^{3}$ /µl)	1.21±1.03^{a}	1.11±0.96^{a}	1.24±0.87^{a}	0.98±0.87^{a}	3.02±0.64^{b}

a,b: Means with different letter as super scripts within a row differ significantly

haematology analyzer may often mis-interpret nRBCs as lymphocytes resulting in inaccurate automated differential leucocyte counts. Neutropenia had been previously documented in *B.gibsoni* infections, the etiology remains unknown and the postulated potential causes were sequestration in capillary beds, increased destruction or utilization and reduced granulopoiesis in bone marrow (Benjamin, 2013; Vishnurahav *et al*., 2014; Brown *et al*., 2015). Significant leucopenia, neutropenia along with lymphocytosis and monocytosis were reported by Reddy *et al*. (2016) in canine babesiosis. However, there was no significant difference in monocyte count in the present study. Nonetheless, monocytic cytoplasmic vacuolation was noted in most of the infected animals indicating an exciting or overwhelming cause disturbing the phagocytic cells.

Variable levels of thrombocytopenia were observed in the current study as reported by Bilwal *et al*. (2017) and Thomas *et al*. (2019). Thrombocytopenia is a most consistent haematological finding in babesiosis which may be often severe, although without apparent clinical effect (bleeding diatheses). It could be due to platelet - leucocyte aggregation, immune mediated destruction or splenic sequ estration or local or systemic intravascular coagulopathy or coagulatory consumption of platelets or concurrent ehrlichiosis (Kettner *et al*., 2003; Reddy *et al*., 2016). The presence of macroplatelets or giant platelets could be observed in blood smear of infected cases which could be suggested as a regenerative response to thrombo cytopenia as described by Simoes *et al.* (2011).

SUMMARY

The study focused on just the tip of the iceberg, the clinical cases of babesiosis and the remaining bulk or the significant proportion of subclinical/chronic status of infection was not however, unveiled. The use of molecular techniques like PCR would have exposed the much more severe and clear-cut status of the infection. Nevertheless, by the use of blood smear examination, a vast canine population over a long period of time could be screened and studied. A predominant prevalence of pathogenic small *Babesia, B. gibsoni*, was noted in the canines of the district over a one-year study period. Haematological findings of regenerative anaemia and thrombo-cytopenia were also noted in concordance with the previous reports. Further, this study calls for the formulation epidemiological patterns, exploration of vector status of the disease, further trials to validate novel treatment modules and genotype variations.

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