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ORIGINAL ARTICLE

Hematological alterations in large *Babesia* species infection in dogs of Kannur District of Kerala

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Abstract Canine babesiosis is a tick-borne disease caused by apicomplexan intraerythrocytic hemoprotozoan parasites. It is caused by the small (*Babesia gibsoni*, *B. conradae*, and *B. vulpes*) and large (*B. vogeli*, *B. canis*, and *B. rossi*) *Babesia* groups. As per the recent reports, the most prominent *Babesia* species encountered in the Kerala state are the small *Babesia, B. gibsoni* followed by the large *Babesia, B. vogeli.* The latter is regarded as mildly pathogenic, causing subclinical or mild disease; however severe complications like systemic inflammatory response syndrome, multiple organ dysfunction syndrome, etc. have also been reported. The information on the status of hematological alterations in naturally infected dogs with large *Babesia* is lacking, particularly from the state of Kerala. The present study involves a retrospective study of clinical cases of large *Babesia* infection in dogs. The complete haematological profile from well-documented laboratory records of 4039 dogs suspected for babesiosis presented to District Veterinary Centre, Kannur during the period from December 2018 to October 2020 was analyzed for the study. Natural infections were recorded in 35 (0.87%) dogs based on the presence of intraerythrocytic piroplasm of large *Babesia* spp. by light microscopic examination of Giemsa-stained blood smears. The most consistent features observed were mild to moderate regenerative, normocytic and normochromic anemia, lowered to normal neutrophil count and thrombocytopenia. In comparison to hemolytic anemia, thrombocytopenia was the most frequent clinicopathological finding in the study with an increased presence of large activated platelets or macro-platelets.

Keywords Anaemia · Thrombocytopenia · Activated platelets · Macroplatelets · Giemsa

Introduction

Babesiosis is a tick-borne disease that occurs worldwide and is caused by a hemoprotozoan belonging to the genus *Babesia* affecting livestock and companion animals (Boozer and Macintire 2003). It was first recognized in 1888 by Babes as a cause of hemolytic anaemia and mortality in cattle (Babes 1888). There are many *Babesia* species affecting dogs that are morphologically classified as large and small forms. The large *Babesia* spp. which infects dogs are *Babesia vogeli*, *B. canis* and *B. rossi;* and the small *Babesia* spp. affecting dogs are *B. gibsoni, B. conradae*, *B. vulpes*, and *B. annae* (Vishwakarma and Nandini 2019). Among the large *Babesia* spp, mildly pathogenic *B. vogeli* transmitted by *Rhipicephalus sanguineus* is reported from Africa, Asia, North America, Brazil and Australia; moderately pathogenic *B. canis* transmitted by *Dermacentor variabilis* is widespread in Europe; and the most virulent and fatal *B. rossi* transmitted by *Haemaphysalis ellipticus* is restricted to sub-Saharan Africa and South Africa (Schoeman 2009). Molecular survey on babesiosis revealed the presence of only two species viz., *B. gibsoni* and *B. vogeli* among canines of the state of Kerala, and the tick population involved in the disease transmission includes *Rhipicephalus sanguineus*, *R. haemaphysaloides* and *H. bispinosa* (Augustine et al. 2017). The latter is regarded as mildly pathogenic causing subclinical or mild disease with atypical clinical presentation, however, can cause severe

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complications like systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), refractory hypotension, septic shock, acid-base ion imbalances, hormonal and renal abnormalities. Clinical findings that may occur are similar to other *Babesia* spp. which includes depression, weakness, anorexia, fever, anaemia, thrombocytopenia, jaundice, lymphadenomegaly, splenomegaly, etc. (Wang et al. 2018). There is scarce published data on the hematological alterations in naturally large *Babesia* infected dogs particularly from the state of Kerala. Hence, the study was conducted to ascertain the hematological variations due to large *Babesia* infection in dogs of the Kannur district of Kerala.

Materials and methods

The present study involved a retrospective analysis of the clinical cases of large *Babesia* infection in dogs. The study was based on well-documented laboratory records on the complete haematological profile of those cases that were tested positive for large *Babesia* on blood smear examination. Laboratory records from December 2018 to October 2020 of the Regional Clinical Laboratory, District Veterinary Centre, Kannur, Kerala, were considered in this study. Dogs that had other diagnosed concurrent diseases or incompletely documented laboratory records were not included in the study. The data regarding signalment, clinical signs and hematological findings were analyzed during the present study. All dogs included in the present study were more than 2 months of age and their ages were documented as per the data provided by the owner. In addition, dogs were divided into two age groups: below 1 year and above 1 year.

A diagnosis of babesiosis was made by finding the piroplasms of large *Babesia* spp. on a thin blood film, stained with Giemsa stain (Himedia, India) and examined under 100× magnification by light microscopy. Around 2 mL of blood was collected from cephalic vein/saphenous vein/recurrent tarsal vein in EDTA vials. Haematological analysis was performed using an automatic haematology analyzer (Exigo EOS, Sweden). Dogs were classified based on the hemoglobin values viz. severely anaemic (Hb < 5 g/dL), moderately anaemic (Hb between 5 and 10 g/dL), mildly anemic (Hb between 10 and 12 g/dL), and non-anaemic (Hb > 12 g/dL). Dogs were also classified based on the platelet values viz. severe thrombocytopenia (less than 1 lakhs/cu.mm), mild thrombocytopenia (1–2 lakhs/cu.mm.), and non-thrombocytopenia (> 2 lakhs/cu.mm). The data obtained were represented as mean ± standard deviation. The parameters of affected animals were compared with those of healthy dogs (n = 10) that visited the hospital for vaccination over the same period of time. 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. Variables with $p < 0.05$ were considered as statistically "significant" and variables with $p > 0.05$ as statistically "non-significant."

Results and discussion

The present study included 4039 dogs of various breeds and age groups presented from December 2018 to October 2020 with different clinical conditions at the outpatient ward of District Veterinary Centre, Kannur (n = 11.8745° N, 75.3704° E). Laboratory records from the Regional Clinical Laboratory, District Veterinary Centre, Kannur, Kerala were used for collecting haematological details of the infected dogs. When the records of 4039 dogs were analyzed, 35 animals (0.87 %) were detected positive for the presence of teardrop shaped piroplasms of large *Babesia* by blood smear examination (Fig. 1).

Records of infected animals when analyzed, the most represented dog breed was Labrador (37.14 %), and least represented breeds were Boxer, Golden Retriever and Beagle (2.86 % each) as depicted in Fig. 2. The exotic breeds described in the study may not be necessarily purebreds as the nature of the pedigree was not known by many dog owners in the state of Kerala due to the lack of knowledge on pedigree systems. In addition, these exotic breeds or their crossbreds made up the largest proportion of dogs affected with large *Babesia*, compared to non-descript (mongrel) dogs.

Rawangchue and Sungpradit (2020) stated that the attributes like sex, breed, clinical signs and geographic origin of the dog have no association in babesiosis. In the present study, more male dogs were infected with large *Babesia* than females (Fig. 3). Obeta et al. (2020) stated that male dogs were 1.24 times more prone to *Babesia* infection than female dogs. Similarly, Salem and Farag (2014) also reported that this might be attributed to increased roaming behavior, sex-linked genetic influences, temperament, and hormonal status of the male dogs. On the contrary, previous studies also reported a higher prevalence of hemoprotozoans in female dogs due to their sedentary nature during nursing periods and immunosuppressive stressful reproductive activities (Jegede et al. 2014; Opara et al. 2017).

In the study, dogs below one year were found more infected than dogs above 1 year of age (Fig. 3). Earlier studies have shown that dogs of all ages are affected with babesiosis, however, young puppies were more affected

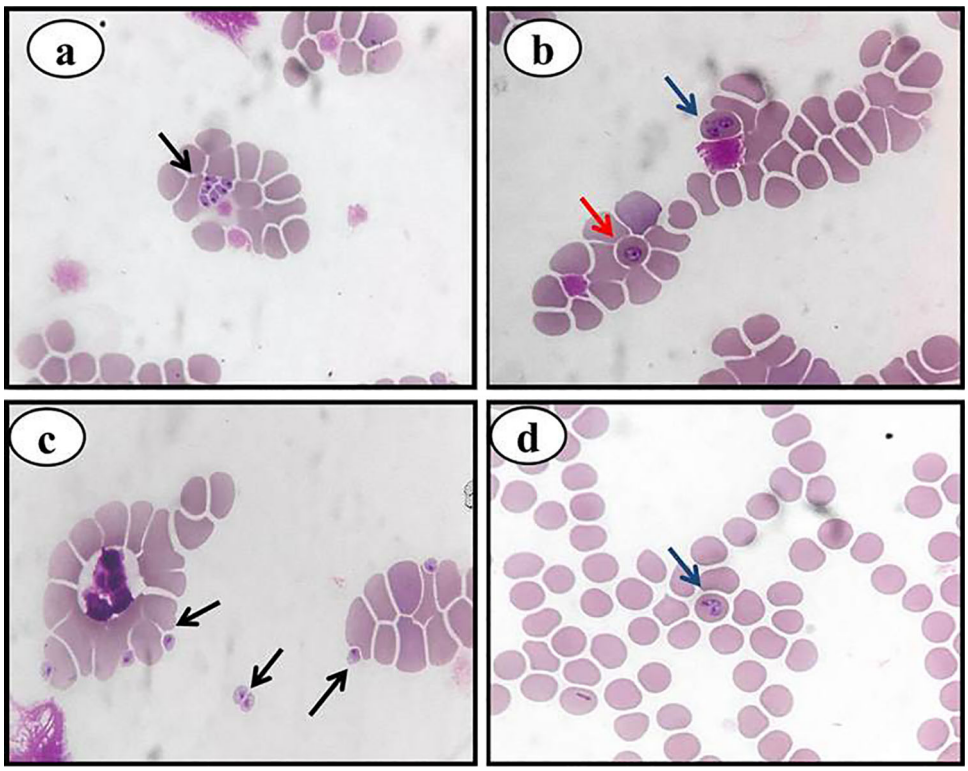

Fig. 1 **a** Babesial piroplasms found in the background (black arrow), which would have probably released from the erythrocyte during smear preparation; **b** Intra-erythrocytic *Babesia* merozoite in the same Giemsa-stained smear (×1000) showing paired or tear shaped pyriform bodies with basophilic cytoplasm and reddish chromatin (blue arrow) and single round piroplasm (red arrow) with macroplatelets and regenerative erythrocytic changes like polychromatophils, macrocytes etc.; **c** Released piroplasms in background can be confused for inclusions in platelets or other blood parasites like *Anaplasma platys*; **d** Paired piroplasms in a non-anemic dog (color figure online)

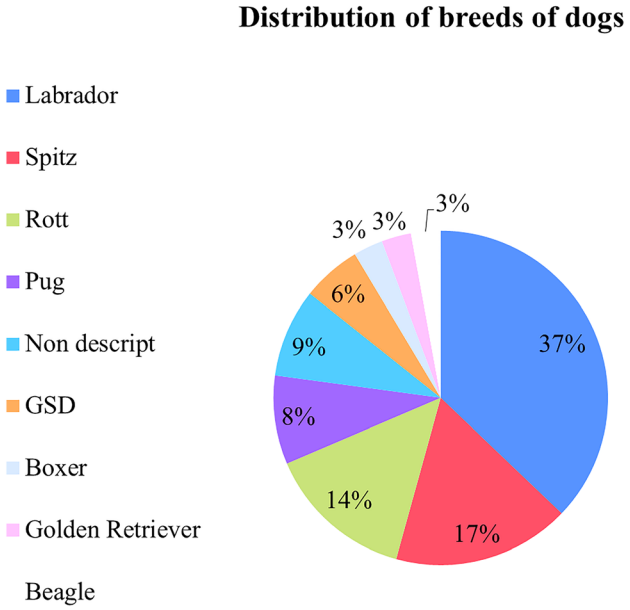

Fig. 2 Distribution of breeds of dogs included in this study

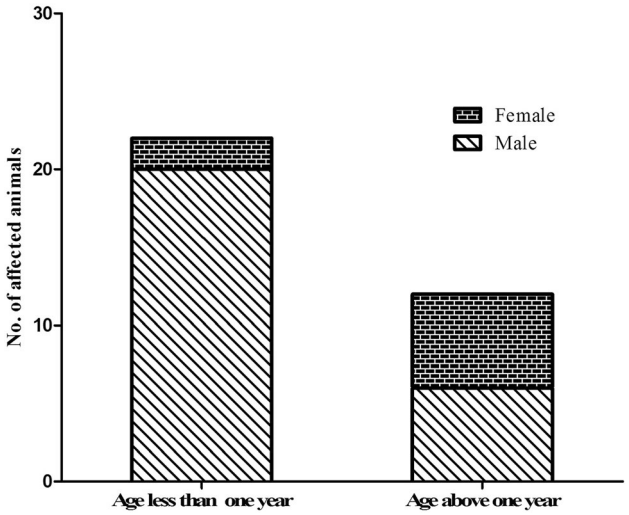

Fig. 3 Age and gender distribution of the affected dogs included in the study

even as young as 3 weeks (Ogo et al. 2011). In another study, a higher incidence of canine babesiosis was found in the age group of > 1– 2 years and explained that this could be due to the underdeveloped immune system (Mahalingaiah et al. 2017). Egege et al. (2008) reported a higher incidence in the age group of 1–3 years attributed to lowered maternal immunity in adult dogs, as well as frequent tick bite exposures.

All dogs in the current study showed reduced feed intake while occasional fever and depression (60% of cases), vomiting (50%), tick infestation (40%), and diarrhoea (2%) were also noticed. Similar clinical signs and presenting problems of anorexia, depression, fever, etc. were reported in *B. vogeli* infection by Nalubamba et al. (2015). Most of

the dogs in the study were asymptomatic or with mild nonspecific symptoms. Previously, Beck et al. (2009) reported similarly that the higher prevalence of the parasite was observed in asymptomatic dogs (7 %) versus symptomatic dogs (1.3 %). The uncommon finding of diarrhoea might be due to the gut form of babesiosis associated with complicating conditions like acute pancreatitis (Mohr et al. 2000).

The haematological findings in blood samples of both infected and healthy animals are shown in Table 1. The most consistent features observed were mild to moderate regenerative, normocytic and normochromic anemia, and thrombocytopenia. Most of the affected animals showed normal Hb count compared to severely anemic animals indicating the mild pathogenicity of the organism (Fig. 4). The anemia may be due to multiple causes like extravascular and intravascular hemolysis, increased erythrocytic osmotic fragility, shortened RBC life span, erythrophagocytosis, and immune-mediated RBC destruction as a result of parasitic antigens, parasite-induced membrane damage and possibly other membrane-associated antigens (Boozer and Macintire 2005). Impaired haemoglobin function, oxidative damage, sludging, and sequestration of erythrocytes may also occur (Vishwakarma and Nandini 2019). Even though Hb and RBC values were low in affected animals, hematocrit values were observed within the normal range. Despite the vigorous hemolysis, few dogs revealed high haemocrits (relative haemoconcentration) which might be due to presumed shifting of fluid from the intravascular to the extravascular component (Welzl et al. 2001). Neutrophil counts were either normal or decreased as observed in the present study; however, left shifts or leukemoid responses were also previously reported (Lobetti 1995).

Unlike hemolytic anemia, thrombocytopenia was identified as the most frequent clinical-pathological finding in the study (Fig. 4). Although 71.43 % infected dogs presented with severe depletion of platelets from circulated blood (< 1 lakh/μL), none of them developed haemorrhage or uncontrollable bleeding. Thrombocytopenia could be due to immune-mediated platelet destruction, platelet sequestration in the spleen, and consumptive coagulopathy like the development of disseminated intravascular coagulation (Boozer and Macintire 2003). Decreased platelet production is considered less likely in canine babesiosis due to increased MPV, which suggested platelet regeneration from bone marrow. Pseudo-thrombocytopenia may also occur due to platelet clumping or adherence of platelets to other blood cells, such as red cell membranes reported in falciparum malaria (Zvorc et al. 2010; Goddard et al. 2015). An increased proportion of macroplatelets was seen in all the infected animals; however, a significant level of MPV was not detected in the study. On the contrary, it was proposed that large, activated platelets were significantly increased in canine babesiosis to conserve the functional platelet mass, demonstrated by increased MPV, mean platelet mass, and decreased mean platelet component concentration (MPC), partially explaining lack of bleeding tendencies in the face of severe thrombocytopenia. Large (immature) platelets are considered functionally more active with a lower threshold for aggregation and release activity (Goddard et al. 2015).

Table 1 Haematological findings in blood samples from large *Babesia* positive dogs (n = 35) and healthy dogs (n = 10)

Parameters	Infected dogs	Healthy dogs
Hb (g/dL)	11.69 ± 3.27^{a}	14.25 ± 1.69^{b}
PCV (%)	34.09 ± 9.66^{NS}	37.11 ± 9.35^{NS}
RBC (millions/ μL)	5 ± 1.47^{a}	6.53 ± 0.71^{b}
MCV (fL)	67.05 ± 15.65^{NS}	66.37 ± 6.48^{NS}
MCH (pg)	23.09 ± 3.24^{NS}	22.13 ± 2.25^{NS}
MCHC (g/dL)	32.82 ± 2^{NS}	33.45 ± 0.42^{NS}
TC ($\times 10^3$ /μL)	8.82 ± 5.7^{a}	13.17 ± 3.14^{b}
NP (%)	62.17 ± 11.3^{a}	70.8 ± 5.41^{b}
LC (%)	27.26 ± 10.29^{NS}	20.7 ± 5.42^{NS}
MC (%)	10.57 ± 3.53^{NS}	8.5 ± 2.17^{NS}
Platelet ($\times 10^3$ /μL)	0.82 ± 0.7^{a}	3.27 ± 0.93^{b}
MPV (fL)	9.1 ± 1.65^{NS}	8.95 ± 1.35^{NS}

Different superscripts indicate mean value differ significantly at $P < 0.05$. Superscript 'NS' indicate mean value does not differ significantly at $P < 0.05$

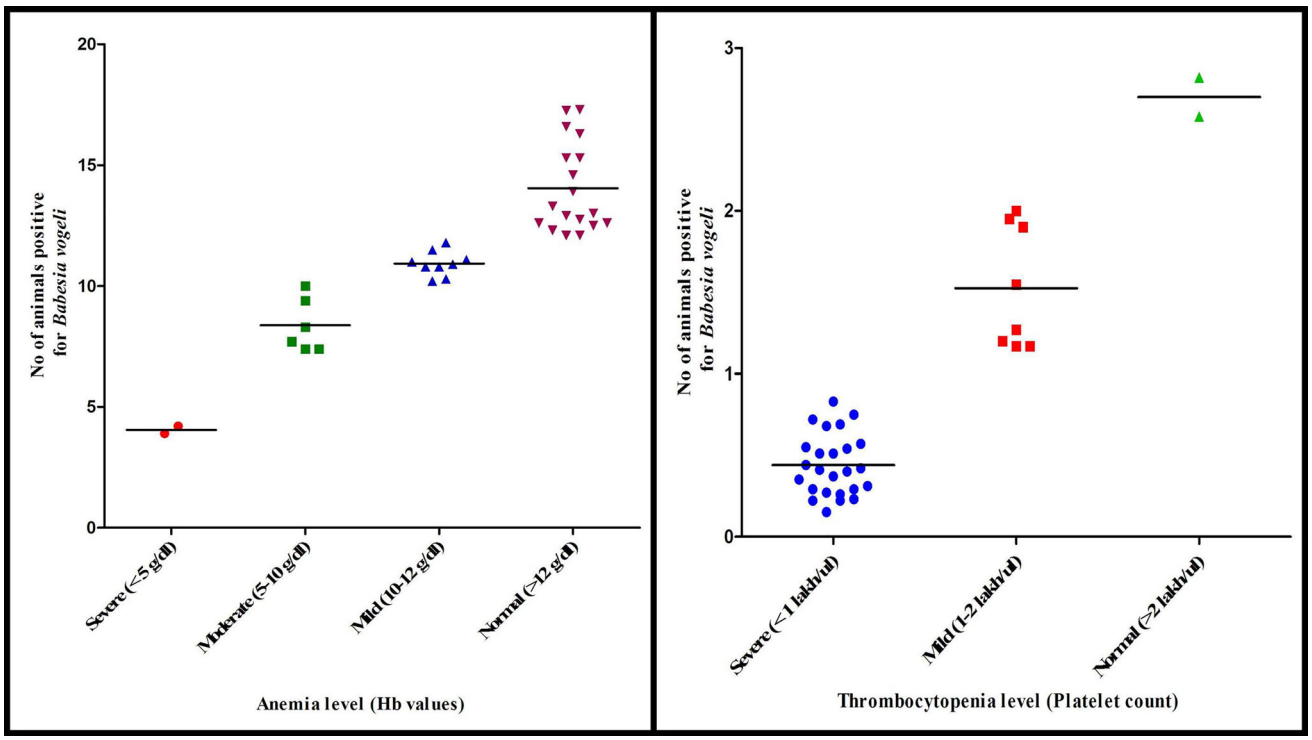

Fig. 4 Severity of anaemia and thrombocytopenia in large *Babesia* infected dogs

It can be concluded that the most consistent findings of clinical canine babesiosis caused by large *Babesia* species were severe thrombocytopenia with a normal or mildly lowered Hb value in the present study. The infections were mostly asymptomatic or atypical and observed more commonly in male dogs and young animals below one year of age. In addition, it was observed that the parasitaemia in infections caused by large *Babesia* is very low; hence, the infection may frequently be missed during the routine blood smear examination, as similarly reported by Irwin and Hutchinson (1991). Hence, it could also be recommended that the results of blood smear examination may also be complemented with other confirmatory or advanced molecular tests to know the exact prevalence of the parasite in asymptomatic canines. The present study identified the occurrence of large *Babesia* infection based on the morphological characteristics of the piroplasm in the blood smear; however, it failed to identify the specific *Babesia* species infecting the dogs. The molecular study to differentiate the different large forms of Babesia spp. affecting dogs is beyond the scope of the current study. In order to differentiate, confirm or rule out the presence of the different large forms of *Babesia* spp. like *B. vogeli, B. canis* and *B. rossi* in India affecting dogs, an extensive epidemiological survey utilizing molecular techniques will be of great help.

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Author contributions PP-performed the laboratory work and prepared the manuscript; SBS, KVRK & SS-involved in collection of samples; SR-provided the required facilities for the work.

Declarations

Conflict of interest There is no conflict of interest for the research findings. There is no separate funding for this research project. The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

Ethical approval Not applicable. Authors collected the blood samples as a part of routine clinical works in the outpatient ward of the hospital.

References

Augustine S, Sabu L, Lakshmanan B (2017) Molecular identification of *Babesia* spp. in naturally infected dogs of Kerala, South India. J Parasit Dis 41(2):459–462

Babes V (1888) Sur l'hemoglobinurie bacterienne du boeuf [Bacterial hemoglobinuria in cattle]. Rend Acad Sci Ser Ser III Vie 107:692–694

Beck R, Vojta L, Mrljak V, Marinculić A, Beck A, Živičnjak T, Cacciò SM (2009) Diversity of *Babesia* and *Theileria* species in

symptomatic and asymptomatic dogs in Croatia. Int J Parasitol 39(7):843–848
Boozer AL, Macintire DK (2003) Canine babesiosis. Vet Clin North Am Small Anim Pract 33(4):885–904
Boozer L, Macintire D (2005) *Babesia gibsoni*: an emerging pathogen in dogs. Compendium 2:33–42
Egege SC, Okolocha EC, Nwanta JA, Mosimabale EO (2008) Prevalence and seasonality of babesiosis in dogs treated at University Veterinary Clinic in Kaduna Nigeria from 1990–1999. Niger Vet J 29(3):21–26
Goddard A, Leisewitz AL, Kristensen AT, Schoeman JP (2015) Platelet indices in dogs with *Babesia rossi* infection. Vet Clin Pathol 44(4):493–497
Irwin PJ, Hutchinson GW (1991) Clinical and pathological findings of *Babesia* infection in dogs. Aust Vet J 68:204–209
Jegede OC, Obeta SS, Faisal B (2014) Infection of dogs with *Babesia canis* in Gwagwalada area of Federal Capital Territory, Abuja. Niger. Sokoto J Vet Sci 12:37–41
Lobetti RG (1995) Leukaemoid response in two dogs with *Babesia canis* infection. J S Afr Vet Assoc 66(3):182–184
Mahalingaiah MKC, Asoor M, Timmaiah RP, Narayanaswamy HD, Mukartal SY, Elattuvalappil AM, Chikkahonnaiah N, Gupta S, Singh S (2017) Prevalence of canine babesiosis in different breeds of dogs in and around bengaluru. Adv Anim Vet Sci 5(3):140–144
Mohr AJ, Lobetti RG, Van der Lugt JJ (2000) Acute pancreatitis: a newly recognised potential complication of canine babesiosis. J S Afr Vet Assoc 71(4):232–239
Nalubamba KS, Mudenda NB, Namwila MM, Mulenga CS, Bwalya EC, M'kandawire E, Saasa N, Hankanga C, Oparaocha E, Simuunza M (2015) A study of naturally acquired canine babesiosis caused by single and mixed *Babesia* species in Zambia: clinicopathological findings and case management. J Parasitol Res 2015: 985015
Obeta SS, Ibrahim B, Lawal IA, Natala JA, Ogo NI, Balogun EO (2020) Prevalence of canine babesiosis and their risk factors among asymptomatic dogs in the federal capital territory, Abuja, Nigeria. Parasite Epidemiol Control 11:e00186
Ogo NI, Lawal AI, Okubanjo OO, Kamani J, Ajayi OO (2011) Current status of canine babesiosis and the situation in Nigeria: a review. Niger Vet J 32:69–78
Opara M, Adewumi N, Mohammed BR, Obeta SS, Simon MK, Jegede OC, Agbede RIS (2017) investigations on the haemoprotozoan parasites of Nigerian local breed of dogs in Gwagwalada Federal Capital Territory (FCT), Nigeria. J Parasitol Res 10:1–7
Rawangchue T, Sungpradit S (2020) Clinicopathological and molecular profiles of *Babesia vogeli* infection and *Ehrlichia canis* coinfection. Vet World 13(7):1294
Salem NY, Farag HS (2014) Clinical, hematologic, and molecular findings in naturally occurring *Babesia canis vogeli* in Egyptian dogs. Vet Med Int 2014:270345
Schoeman JP (2009) Canine babesiosis. Onderstepoort J Vet Res 76(1):59–66
Vishwakarma P, Nandini MK (2019) Overview of canine babesiosis. In: Veterinary medicine and pharmaceuticals. IntechOpen. https://doi.org/10.5772/intechopen.82243
Wang J, Zhang J, Kelly P, Zheng X, Li M, You J, Huang K, Qiu H, Wang Y, Zhang R, Li J (2018) First description of the pathogenicity of *Babesia vogeli* in experimentally infected dogs. Vet Parasitol 253:1–7
Welzl C, Leisewitz AL, Jacobson LS, Vaughan-Scott T, Myburgh E (2001) Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. J S Afr Vet Assoc 72(3):158–162
Žvorc Z, Barić Rafaj R, Kuleš J, Mrljak V (2010) Erythrocyte and platelet indices in babesiosis of dogs. Veterinarski arhiv 80(2):259–267