



Prevalence and Role of Anemia in Mortalities of Dogs with Babesiosis

Simon Abah Victor Abakpa^{1,*}, Esther Inioluwa Mustapha¹, Fakilahyel Musa Mshelbwala¹, Kemi Ruth Idowu², Mary Idowu Olasoju¹, Olukayode Gbolahan Akintunde¹, Edwin Favour Okpe¹, Temiloluwa John Fambege¹, and Olajoku Jokotola Awoyomi¹

¹College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria

²College of Animal Sciences and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria

*Corresponding author's Email: abakpasav@funaab.edu.ng

ABSTRACT

Anemia is a decrease in red blood cells (RBC), packed cell volume (PCV), and hemoglobin in the blood due to hemolysis, hemorrhage, or decreased production of RBC. This research determined the prevalence, characteristics, and role of anemia in association with babesiosis in dogs for one year. A total of 103 dogs positive for babesiosis presented at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria, were screened for anemia. Among these, 80 dogs displaying anemia were selected. A thin blood smear was used to identify *Babesia* spp. The PCV and hemoglobin concentration (Hb) were determined using an automatic hemoglobinometer (Acon Laboratories, Inc., San Diego, USA). Temperature, pulse, PCV, Hb, and RBC assessed were correlated with mortalities. The findings indicated that 32 anemic babesiosis were male. The percentage of severe anemia was higher, compared to mild or moderate anemia. The age-specific prevalence of anemia was highest among dogs above 3 years but lowest in the age range of 1-3 years. Small breeds of dogs had a higher prevalence of anemia compared with larger breeds. The mortality rate was 18 (22.5%), with severe or microcytic hypochromic anemia being the highest. The temperature and pulse rates of the dogs that died were higher than those of survivors, while the PCV, Hb, and total RBC counts were significantly lower than those of survivors. In conclusion, the prevalence of anemia in dogs with babesiosis in this study was very high. Most of the mortalities recorded in the present study can be attributed to severe anemia and microcytic hypochromic anemia, with mortality rates of 61.1% and 38.9%, respectively.

Keywords: Anemia, Babesiosis, Dog, Mortality, Prevalence

INTRODUCTION

Babesiosis is a tick-borne parasitic infection (Irwin, 2009) caused by hemotropic protozoa of the genus *Babesia* spp. with a worldwide distribution and global significance (Turna et al., 2022; Helm et al., 2022), affecting both humans and animals (Bajer et al., 2022). The disease occurs in all ages of dogs, although there seems to be a higher incidence in younger dogs (Nalubamba et al., 2011). *Babesia* spp. responsible for the infection have been classified into large (*Babesia* [*B.*] *canis*) and small *babesia* (*B. gibsoni*). Molecular characterization further divided the large *babesia* into three subspecies (*B. canis canis*, *B. canis rossii*, and *B. canis vogeli*), which are morphologically identical (Skotarczak, 2008). The subspecies of *B. canis* were earlier classified based on their antigenic properties, pathogenicity, and geographic distribution (Uilenberg, 2006). However, they were recently assigned to the categories *B. canis*, *B. rossii*, and *B. vogeli* (Yisaschar-Mekuzarset et al., 2013). The small *babesia* of dogs originated from North American and Asian countries, spread to other parts of the world (Teodorowski, 2020), and belong to different genetically distantly related species (Zahler et al., 2000). While *B. rossii* is one of the most pathogenic species, *B. canis* is the most common cause of babesiosis (Konvalinova et al., 2012). In Nigeria, *B. canis rossii* (Takeet et al., 2017) and *B. canis vogeli* (Sasaki et al., 2007; Adamu et al., 2014) have been reported in dogs. *Babesia* spp. primarily parasitizes erythrocytic cells, causing intra-erythrocytic parasitemia, resulting in both intravascular and extravascular hemolysis, ultimately resulting in hemolytic anemia.

Anemia is a clinical and laboratory sign, not a disease resulting from different primary conditions and diseases (Giger, 2005; Yadav et al., 2022). Anemia can be seen in splenic relaxation in cases of anesthetic agent use, resulting in sequestration of RBC (up to 30%) in the spleen. Physiologic anemia is possible in young animals as a result of rapid growth rate with hemodilution from plasma expansion volume, dilution from ingested colostrum, destruction of fetal RBC, or decreased production due to low erythropoietin concentration during the first few weeks of life (Cowgill et al., 2003). Different factors, such as chronic bleeding, malabsorption, or chronic inflammation, could also be responsible for

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anemia. Clinical practices in humans have revealed that iron deficiency anemia and the combined forms of anemia due to different pathophysiological mechanisms are the most common anemia (Tomasević et al., 2022).

The common causes of anemia in dogs in Nigeria have been attributed to parasitic infestations and hemoparasitic infections, with nutrition contributing less to the cause (Useh et al., 2003). *Ehrlichia canis*, *B. gibsoni*, primary immune-mediated hemolytic anemia, neoplasia, *B. canis*, and toxicity have been reported to be the most common cause of anemia in Ludhiana, in that order (Bhat et al., 2016). The most commonly observed clinical signs in dogs are weakness, depression, pale mucous membrane, inappetence or anorexia, and reduced activities (Useh et al., 2003). Severity, RBC indices, and regenerative response generally characterize anemia. The severity of anemia is determined by the degree of decrease in the Packed Cell Volume/Hematocrit, which varies from species to species, depending on the lower limit of the reference interval or reference value for the severity of the anemia in dogs as 30-36% (mild) 20-29% (moderate) and < 20% (severe) (Soundarya and Suganthi, 2016). The present study determined the prevalence, characteristics, and severity of anemia and its association with mortalities in dogs with babesiosis, presented at the Veterinary Teaching Hospital (VTH), Federal University of Agriculture, Abeokuta, Nigeria.

MATERIALS AND METHODS

Study design

A cross-sectional survey was used in this study in which 103 dogs presented at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria, were randomly sampled. Dogs sampled were divided into three age groups comprising <1 year, 1-3 years, and > 3 years.

Procedure

The rectal temperature and pulse rates of dogs presented at the veterinary teaching hospital were determined before the physical examination. Three milliliters of blood were taken from each dog's cephalic vein into bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) for determination of packed cell volume (PCV), hemoglobin concentration, and parasite detection. Fecal samples were taken and examined for the absence of gastrointestinal parasites immediately after collecting using the simple flotation technique. A total of 986 dogs presented to the Small Animal Clinic, out of which 103 were positive for babesiosis and were recruited for this study. Dogs positive for ehrlichiosis, trypanosomosis, isosporosis, or gastrointestinal parasite infections, dogs with a history of bleeding or malnutrition were excluded from the study, and just 80 dogs were considered in this study. Pregnant bitches were also excluded from this study.

Identification of *Babesia* spp. using thin blood smear

A drop of uncoagulated blood was dropped on one end of a clean glass slide, and another glass slide was placed at an angle of about 300-450 to touch the blood, allowing it to spread along the contact line of the slide, and a thin blood smear was made. The thin blood smear was air-dried and fixed by dipping the slide into a container containing methanol for 30 seconds. Staining was done using Giemsa stain, and the stained blood smear was allowed to dry for three minutes. The samples were observed at 100× magnification under a light microscope (Olympus®, Japan) to identify the parasite (*Babesia* spp), which is pyriform in shape and found within the red blood cells.

Determination of the packed cell volume, hemoglobin concentration, and erythrocyte indices

The packed cell volume and hemoglobin concentration were determined by the use of an automated hemoglobinometer. The hemoglobinometer was put on, and the strip was inserted. A drop of blood was added to the demarcated area when a blue light appeared. The readings were displayed on the digital surface of the hemoglobinometer. Erythrocyte indices were determined using the following Formula 1. The PCV, hemoglobin concentration, and red blood cell concentration (erythrocyte indices) were used to calculate the mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) and used to determine the type of anemia.

$MCV = PCV/RBC \times 10 \text{ fl}$, $MCH = Hb/RBC \times 10\text{pg}$, $MCHC = Hb/PCV \times 100\text{g/dl}$. (Formula 1)

The values of MCV less than 80 fl, 80-94 fl, and greater than 94 fl were considered microcytic normocytic and macrocytic anemia, respectively (Yadvet et al., 2021). Briefly, 30-36% of anemia was considered mild, 20-29% moderate, while less than 20% was severe. The severity of anemia was determined as described by Soundarya and Suganthi (2016).

Data analysis

Data generated were analyzed using descriptive statistics with the statistical software IBM SPSS Version 20. The chi-square test and independent t-test were used to determine the differences between variables. Odds ratio with a 95% confidence interval (CI) was calculated for the association between certain variables and anemia, and statistical differences at the level of $p < 0.05$ were considered significant.

RESULTS

Prevalence of anemia

Out of the 103 dogs infected with *babesia*, 41 (39.8%) were male, while 62 (60.2%) were female. Moreover, a total of 80 (77.7%) dogs exhibited anemia, with 32 (40%) being male and 48 (60%) being female. The mortalities recorded in the current study were 18 (17.5%) and were only seen among the 80 anemic dogs, Table 1). The highest percentage of anemic dogs was recorded under mild anemia 30 (37.5%), followed by moderate anemia 26 (32.5%). Those that had severe anemia were 24 (30.0%). Anemia was insignificantly higher in male than female dogs ($p > 0.05$). Among the age groups, the percentage of anemia was higher in dogs above 3 years. Similarly, it was higher in small breeds than in large breeds ($p > 0.05$, Table 1).

Classification of anemia

Among the anemic dogs, the mortality rate was 18 (22.5%). The observed pattern in this study revealed that severe anemia accounted for the highest percentage of deaths, totaling 11 (61.1%) among the deceased dogs. On the other hand, mild and moderate anemia cases constituted 5 (27.8%) and 2 (11.1%) of the deaths, respectively. In terms of morphology, normocytic hypochromic anemia exhibited the highest prevalence at 45 (56.3%) cases, and it was associated with a mortality rate of 4 (22.2%). In contrast, normocytic hyperchromic anemia was the least prevalent in 2 (2.5%) cases. The highest number of deaths, 7 (38.9%), was observed in dogs with microcytic hypochromic anemia, closely followed by macrocytic hyperchromic anemia at 6 (33.3%). There were no reported instances of mortality among dogs with normocytic hyperchromic anemia (Table 2).

Relationship between health indices and mortality

The mean temperature of the dead dogs at the time of referral was $39.4 \pm 0.2^\circ\text{C}$, while that of the survivors was $39.2 \pm 0.10\text{C}$, with that of the dead being slightly higher than that of the survived ones. There was no significant difference ($p > 0.05$) between the mean temperature of those that died and those that survived. The mean pulse rate of dogs that died was significantly higher than that of the surviving ones. The mean PCV, hemoglobin concentration, and RBCs of dogs that died were significantly ($p < 0.05$) lower than those that survived (Table 3).

Table 1. Prevalence of anemia in dogs with babesiosis at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria

	Variables	Number of cases	Anemic (%)	Non-anemic (%)
Sex	Male	41	32 (78.0)	9 (22)
	Female	62	48 (77.4)	14 (22.6)
Age	< 1 year	40	33 (82.5)	7 (17.5)
	1-3 years	49	33 (67.3)	16 (32.7)
	> 3 years	14	14 (100)	0 (0)
Breed	Large	91	70 (76.9)	21 (23.1)
	Small	12	10 (83.3)	2 (16.7)
Total		103	80 (77.7)	23 (22.3)

No.: Number

Table 2. Severity and morphological classification of anemia recorded in dogs with babesiosis at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria.

	Types of Anemia	No. of cases	Mortality	
			Dead	Survived
Classification	Anemic	80	18 (17.5%)	62
Severity	Mild	30	5 (27.8%)	25
	Moderate	26	2 (11.1%)	24
	Severe	24	11 (61.1%)	13
Characteristics	Microcytic hypochromic	14 (17.5)	7 (38.9%)	7
	Normocytic hypochromic	45 (56.3%)	4 (22.2%)	41
	Normocytic normochromic	5 (6.3%)	1 (5.6%)	4
	Normocytic hyperchromic	2 (2.5%)	0 (0%)	2
	Macrocytic hyperchromic	14 (17.5%)	6 (33.3%)	8
	Total		80	18

No.: number

Table 3. Relationship between health indices and mortalities in dogs with babesiosis at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria

Parameters*	Mortalities	Survival	P – value
Temperature (°C)	39.4 ± 0.2	39.2 ± 0.1	0.434
Pulse Rate (bpm)	127.3 ± 8.0	115.4 ± 1.3	0.013
Packed Cell Volume (%)	16.6 ± 2.8	26.6 ± 1.1	0.001
Hemoglobin Conc (mg/dl)	5.9 ± 0.9	9.2 ± 0.4	0.001
Red Blood Cell (x 10 ⁶)	3.8 ± 0.3	6.7 ± 0.3	0.000

*: The parameters were measured at the time of case presentation.

DISCUSSION

Canine babesiosis is one of the main protozoan diseases usually encountered in Nigeria. It is considered a worldwide cause of hemolytic anemia (Boozer and Macintire, 2003). The prevalence of anemia observed in dogs suffering from canine babesiosis in the present study (77.7%) was higher than that of Bhat et al. (2016), who reported 44.83% in dogs with the same disease. The high prevalence of anemia recorded in dogs with babesiosis in this study suggests that anemia is the hallmark of the disease, which agrees with the report of Fabisiak et al. (2010). In this study, the number of females that were anemic was relatively higher than that of the males; however, it was insignificant. This result showed that anemia in babesiosis is not sex-dependent. Although pregnancy can influence anemia in both health and disease conditions (Brabin et al., 2001), pregnant bitches were excluded from this study.

Anemia has been reported as the hallmark of canine babesiosis resulting from intravascular and extravascular hemolysis (Fabisiak et al., 2010). The high prevalence of anemia, mostly mild, recorded in this study corroborates the report of Fabisiak et al. (2010). Anemia of babesiosis origin is often normochromic to hypochromic (Fabisiak et al., 2010), which was partly evident in this study. Fabisiak et al. (2010) reported that anemia in babesiosis often appears as microcytic hypochromic, and regenerative as the disease progresses after an initial picture of mild, normocytic normochromic anemia. The finding in this study is at variance with a previous report, which presented regenerative, normochromic, and normocytic anemia with thrombocytopenia (Schetters et al., 1988) as the main hematological features during canine babesiosis course. In the current study, the highest percent was microcytic hypochromic anemia, a pointer that iron deficiency may be associated with babesiosis, as Van de Maele et al. (2008) reported. Anemia may be a contributing factor in the mortality of both humans and animals (Zhang et al., 2022). In dogs, anemia can lead to hypoxia, resulting in the alterations of macro- and micro-circulations (Zygner and Gojska-Zygner, 2014), and is reported to be an indicator of poor nutrition and health (WHO, 2014). In the present study, the percentage of mortalities among the anemic dogs was high, and it was observed that 61.1% of those with severe anemia died. This is an indication that dogs with severe anemia in cases of babesiosis would rarely survive. The current findings indicated that all the biomarkers of anemia (RBC count, PCV, and hemoglobin concentration) were very low in the dogs that died, compared to the survivors, showing that the oxygen-carrying capacity of their blood was highly compromised. The higher pulse rate seen in the dogs before death was probably due to the low blood indices prompting the heart to beat above the normal rate to meet the peripheral blood demands.

CONCLUSION

In conclusion, the prevalence of anemia in dogs associated with babesiosis in the present study was 77.7% and characterized mainly by normocytic hypochromic indices. Severe and microcytic hypochromic anemia were responsible for the high percentage of dog mortalities.

DECLARATION

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Competing interests

The authors declared that there are no competing interests.

Ethical consideration

The authors considered the ethical concerns and consent of pet owners before recruiting them for the study. This article was written originally without any copy from other articles.

Authors' contributions

Simon A.V. Aabakpa and Esther S. Mustapha conceptualized, designed, and supervised the research. Musa F. Mshelbwala and Mary I. Olasoju critically reviewed the study. Kemi R. Idowu, Temiloluwa J. Fambegbe, and Edwin F. Okpe were involved in collecting samples and processing the data. Olajoju J. Awoyomi analyzed and interpreted the data generated. All authors revised and approved the final manuscript.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available. The authors of this article hereby confirm that all data supporting the findings of this research are available upon reasonable request.

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