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Diagnostic performance of Giemsa and Field's stain thin blood films for detecting canine babesiosis in Makurdi, Nigeria

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Abstract

Canine babesiosis is an important tick-borne zoonotic disease that constrains dog health and breeding in Nigeria. Reliable low-cost laboratory diagnosis is essential for effective case management and disease control. This study determined the prevalence of canine babesiosis and evaluated the diagnostic performance of Giemsa- and Field-stained thin blood films for microscopic detection of *Babesia* spp. Blood samples were collected from 141 dogs of different breeds in Makurdi, Nigeria, during the dry and rainy seasons. Thin blood films were prepared and stained with Giemsa stain or Field's stain (A and B) and examined microscopically for *Babesia* spp., while packed cell volume (PCV) was determined by the microhematocrit technique. The overall detection rate of canine babesiosis was 13.48%, with both Giemsa- and Field-stained thin blood films detecting all positive cases, and yielded an identical mean parasitaemia score. Mean PCV was significantly lower in infected dogs than in non-infected dogs ($p < 0.05$), indicating the association between infection and anaemia. There were no significant associations observed between babesia infection and sex ($P = 0.566$), breed ($P = 0.725$), age ($P = 0.511$), season ($P = 0.135$, and sampling location ($P = 0.807$). The comparable performance of Giemsa- and Field-stained thin blood films indicates that either staining method can be used for routine microscopic diagnosis of canine babesiosis. Despite equivalent diagnostic sensitivity, Field-stained thin blood films were faster to prepare than Giemsa-stained thin blood films, offering a practical advantage for routine diagnostic laboratories. Given the simplicity, low cost, and accessibility of these techniques, they remain suitable diagnostic tools for veterinary laboratories, particularly in resource-limited settings where advanced molecular methods are not readily available.

Novelty statement

To our knowledge, this is among the few studies in Nigeria to directly compare Giemsa- and Field-stained thin blood films for the microscopic diagnosis of canine babesiosis. The study provides locally relevant evidence that both staining methods yield comparable diagnostic performance, supporting their application in routine veterinary practice in resource-constrained settings.

INTRODUCTION

Canine babesiosis is one of the most important tick-borne haemoprotozoan diseases affecting domestic and wild canids worldwide. The disease is caused by intraerythrocytic protozoan parasites of the genus *Babesia*, which invade and multiply within red blood cells, resulting in haemolytic anaemia, fever, lethargy, haemoglobinuria, jaundice, and, in severe cases, death (Solano-Gallego et al., 2016; Schoeman, 2019; Irwin, 2009). Beyond its veterinary significance, babesiosis is increasingly recognized as a zoonotic disease of public health concern, with several human infections reported in Europe and the United States (White et al., 1998). The growing interaction between humans, domestic animals, and tick vectors has heightened concerns regarding the epidemiological importance of canine babesiosis and its potential implications for both animal and human health.

Among the canine *Babesia* species, *Babesia canis* is one of the most widely distributed and clinically significant. Based on biological characteristics, pathogenicity, vector specificity, and genetic differences, *B. canis* has been subdivided into three subspecies: *Babesia canis canis*, *Babesia canis rossi*, and *Babesia canis vogeli* (Breitschwerdt et al., 1999). These parasites are transmitted primarily through the bite of infected ixodid ticks, particularly species of the genera *Rhipicephalus*, *Dermacentor*, and *Haemaphysalis*. (Irwin, 2009; Vannier & Krause, 2012; Schnittger et al., 2012).

Canine babesiosis remains endemic in many tropical and subtropical regions, including Nigeria, where environmental conditions favour the survival and proliferation of tick vectors. Several studies conducted across different parts of Nigeria have documented the occurrence of canine haemoparasitic infections, with babesiosis consistently reported as one of the most prevalent diseases affecting dogs (Useh et al., 2003; Adamu et al., 2014; Kamani et al., 2019; Ezeokoli et al., 2021). The disease imposes considerable economic burdens on dog owners through treatment costs, reduced productivity of working dogs, and increased mortality. Furthermore, the role of dogs as companion animals and potential reservoirs of tick-borne pathogens underscores the importance of effective diagnosis and control measures.

Accurate and timely diagnosis is essential for the successful management, prevention, and control of canine babesiosis. A wide range of diagnostic approaches have been employed, including epidemiological assessments, clinical evaluation, parasitological examinations, immunodiagnostic assays, and molecular techniques (Garcia, 2007; Solano-Gallego et al., 2016; Schnittger et al., 2020). While molecular methods such as polymerase chain reaction (PCR) and serological assays offer high sensitivity and specificity, their application in many developing countries is often constrained by high costs, limited laboratory infrastructure, and technical expertise requirements. Consequently, parasitological diagnostic methods remain the most widely used laboratory techniques in routine veterinary practice because they are inexpensive, rapid, and relatively easy to perform.

Microscopic examination of stained blood smears remains the cornerstone of parasitological diagnosis of canine babesiosis. Romanowsky-type stains, particularly Giemsa and Field stains, are commonly employed to visualize blood parasites in peripheral blood samples. These staining techniques facilitate the identification of intraerythrocytic protozoa and other blood-borne pathogens, including rickettsial organisms and microfilariae (Cheesbrough, 2006; Blevius et al., 2008; Campbell and Reece, 2020). The simplicity, affordability, and accessibility of microscopy make it particularly valuable in resource-limited settings where advanced molecular diagnostics may not be readily available.

Although both Giemsa and Field stains belong to the Romanowsky family of stains and operate through similar staining mechanisms, differences in their preparation, staining procedures, and physicochemical properties may influence diagnostic performance (Cheesbrough, 2006; WHO, 2022). Giemsa stain is traditionally regarded as the reference stain for haemoparasite diagnosis because of its excellent nuclear and cytoplasmic detail. Field stain, on the other hand, is a rapid water-based Romanowsky stain that is easier to prepare, more stable under field conditions, and requires considerably less staining time (Cheesbrough, 2006; WHO, 2022). These characteristics may enhance its practicality for routine

diagnosis, particularly in field and low-resource laboratory settings. However, variations in staining characteristics could potentially affect parasite visualization and consequently influence diagnostic sensitivity.

In addition to staining methods, the type of blood film preparation may significantly influence parasite detection rates. Thin blood films allow detailed visualization of parasite morphology and species identification, whereas thick blood films increase the concentration of blood elements and may improve the detection of low parasitaemia. Buffy coat preparations further enhance diagnostic sensitivity by concentrating parasitized erythrocytes and other cellular components. Consequently, differences in blood film preparation techniques may contribute to variations in the sensitivity and accuracy of parasitological diagnosis (Schoeman, 2019).

Despite the widespread use of microscopy for the diagnosis of canine babesiosis, there is limited information comparing the diagnostic performance of different combinations of Romanowsky stains and blood film preparations under field conditions in Nigeria. Determining the most sensitive, rapid, and practical parasitological technique is critical for improving disease detection, treatment outcomes, and surveillance efforts. Such information would be particularly valuable in endemic regions where laboratory resources are limited and rapid decision-making is essential.

Therefore, this study was designed to compare two Romanowsky stains, Giemsa and Field's stain, for the microscopic detection of canine babesiosis on thin blood films in Makurdi, North-Central Nigeria, and to examine the influence of selected demographic and seasonal factors on infection. It was hypothesized that variations in blood film preparation and staining methods significantly affect the sensitivity of parasitological laboratory diagnostic techniques for the diagnosis of canine babesiosis. Therefore, the main aim of this study was to compare routine microscopic staining methods for detection of babesiosis in dogs.

METHODS AND MATERIALS

Study design

We conducted a cross-sectional survey between the period of July and December to determine the prevalence of canine babesiosis and associated epidemiological factors in naturally infected dogs within Makurdi Metropolis, Nigeria. A total of 141 client-owned dogs presented at the Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine, University of Agriculture, Makurdi, and at Green Flash Veterinary Services, together with dogs sampled in the field, were included in the study. Blood samples were collected once from each animal and examined using Giemsa- and Field-stained thin blood film techniques for the detection and estimation of Babesia parasitaemia. Epidemiological variables (sex, age, breed, and season) were recorded and evaluated in relation to infection status.

Study Area

Makurdi, the capital of Benue State, is located in North-Central Nigeria. The River Benue runs through the town, separating it into North and South Banks. Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine, University of Agriculture, Makurdi, is located on the North Bank, while Green Flash Veterinary Services is located on the South Bank of Makurdi. Makurdi is located on Latitude 7^0 – 44^0 N and longitude 8^0 – 35^o E (Balogun, 2000). There are two distinct seasons, the rainy season and the dry season. The areas sampled were the North and South Banks of Makurdi.

Ethical consideration

We collected blood samples from client-owned dogs in the field exclusively for research purposes following informed verbal consent from their owners. The sample collection was also performed as part of routine clinical examination and animal care at the Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine, University of Agriculture, Makurdi. Qualified veterinary personnel carried out all procedures in accordance with standard veterinary practices, with due consideration for animal welfare. No additional invasive procedures beyond those required for routine clinical management and diagnostic blood collection were performed.

Determination of Sample Size

The sample size was determined using a formula described by THRUSFIELD (1995).

$N = 1.96^2 P\ exp\ (1 - P\ exp) / d^2$. Where;

N =sample size, d = desired absolute precision of 5% (0.05),

P exp = Expected Prevalence (10.2%)

1. − P exp = 1 − 0.102

= 0.898

The prevalence rate of Babesiosis within Makurdi Metropolis is 10.2% (OMUDU; et al, 2007).

Substituting in the formula, $N = 1.96^2 P\ exp\ (1 - P\ exp) / d^2$.

Thus, $N = 1.962 \text{ X } 0.102(0.898) / (0.05)^2$

N = 0.351875193 /0.002

Thus, N = 140.8, approximately 141.

Thus, the sample size was 141.

Method of Sampling

Blood samples were collected from three breeds of dogs selected from two Veterinary Clinics in Makurdi, Veterinary Teaching Hospital, University of Agriculture Makurdi (North Bank) and Green Flash Veterinary Services (South Bank), and also from homes across the study area. Samples were collected from July to Dec. 2014, cutting across rainy and dry seasons.

Collection of Blood Samples

We collected blood samples according to WHO (2022) and Wayne, P.A (2017). BRIEFLY: The dog was properly restrained and the site of the cephalic vein cleaned with cotton wool soaked in 70% alcohol. A sterile 5ml syringe and hypodermic needle was pierced into the vein and 3ml of blood drawn from the vein of each dog. The blood was transferred to a specimen bottle containing Ethylene Diamine Tetra-acetic acid (EDTA) and conveyed to the Veterinary Teaching Hospital (VTH) laboratory, Faculty of Veterinary Medicine, University of Agriculture, Makurdi using ice packs contained in a flask.

Laboratory Procedures

The 141 blood samples collected were analysed in the Parasitology /pathology Laboratory of the Veterinary Teaching Hospital, University of Agriculture, Makurdi. Laboratory diagnostic techniques used to analyse the samples include Giemsa stain thin blood films, Field stain thin blood films, and haematocrit concentration techniques. Two stains were used: Giemsa stain and Field's stain A and B, which were obtained from the same source (BEMA STANDARD STAIN PREPARED BY BEMA SCIENTIFIC AND CHEMICAL CO.LTD, SHENYANG- CHINA). Thin blood films were prepared based on standard procedures for preparation of blood films (Cheesbroug 2006). The haematocrit concentration technique was used to determine the packed cell volume (PCV). Briefly, thin films were fixed in absolute Methanol for 5minutes. The prepared blood films were stained based on standard procedures for staining thin blood films using Giemsa and Field stains (Cheesbrough 2006).

Data analysis

We summarised our data as counts, percentages, and means ± standard error. We tested the associations between infection status and demographic or seasonal variables using the chi-square (χ^2) test. Mean PCV of infected and non-infected dogs was compared using an appropriate test of means. For analysis, semi-quantitative parasitaemia scores were transformed to numerical values (+ = 1, ++ = 2, +++ = 3) to derive a mean parasitaemia score. A probability of $p < 0.05$ was considered statistically significant.

RESULTS

A total of 141 dogs were examined; 19 were positive for *Babesia spp*, representing a 13.48% prevalence rate (Fig. 1). The overall detection rate of canine babesiosis was 13.48%, with both Giemsa- and Field-

stained thin blood films detecting all positive cases, giving a detection rate of 13.48% (Table 1). Mean PCV was significantly lower in infected dogs than in non-infected dogs ($p < 0.05$) (Table 2), indicating the association between infection and anaemia. No significant associations were observed between babesia infection status and sex, breed, age, season, and sampling location, with p-values of 0.566, 0.725, 0.511, 0.135, and 0.807, respectively (Table 3).

Table 1
Microscopic detection rate and parasitemia scores of Babesia infection in relation to diagnostic techniques

Diagnostic technique	**Number Examined**	**Number Infected**	**% Infected**	**Parasitemia score**	**Mean Parasitemia**
Field stain thin film	141	19	13.48	+ (17)	+ (1.11)
				++ (2)	
				+++(0)	
Giemsa stain thin film	141	19	13.48	+ (17)	+ (1.11)
				++ (2)	
				+++ (0)	

Parasitemia scoring was based on Cheesbrough (2006). Transformation into equivalent numerical mean value where, + = 1, ++ = 2, +++ = ++++ = 4.

Table 2
Packed Cell Volume (PCV) values in relation to *Babesia species* infection in different breeds of dogs.

Breed	**Mean PCV Infected**	**Mean PCV Non-Infected**
Exotic	22.50 ± 3.23^a	35.85 ± 1.72^a
Mixed	19.00 ± 1.87^a	31.33 ± 1.17^a
Mongrel	24.91 ± 3.14^a	32.14 ± 1.06^a

Rows with the same alphabets indicate statistically significant differences

Table 3
Effects of demographic and seasonal factors on Babesia infection

Demographic factor	Variable	Number. Examined	Number Infected	% Infected	df	X^2	P-value
Sex	Female	73	11	15.07	1	0.3296	0.566
	Male	68	8	11.76			
	Total	141	19	13.48			
Breed	Mongrel	82	11	13.41	2	0.644	0.725
	Mixed	22	4	16.00			
	Exotic	37	4	10.81			
	Total	141	19	13.48			
Age	Puppies (0 ≤ 3months)	24	5	20.83	2	1.344	0.511
	Adolescents(> 3 ≤ 12months)	33	4	12.12			
	Adults (> 12months)	84	10	11.90			
	Total	141	19	44.85			
Seasonal variation	Rainy season (15th July to 30th Oct)	100	16	16.00	1	2.240	0.135
	Dry season (1st Nov to 15th Dec).	41	3	7.32			
	Total	144	19	23.32			
Location	North Bank	71	9	12.68	1	0.059	0.807
	South Bank	70	10	14.29			
	Total	141	19	26.97			

DISCUSSIONS

This cross-sectional study screen different breeds of dogs in Makurdi Metropolis, Nigeria, between July and December to evaluate the detection of canine babesiosis using Giemsa and Field stains and to examine the influence of selected demographic and seasonal variables on infection. Among the 141 dogs screened, 19 were positive for *Babesia* spp., giving an overall prevalence of 13.48%. The findings of this study confirm that canine babesiosis remains an important tick-borne disease of dogs in Makurdi Metropolis. The prevalence recorded was slightly higher than that previously reported by Omudu et al.

(2007), a difference that may reflect variations in study design, sampling period, and the use of multiple diagnostic methods, which likely enhanced case detection.

Babesia infection was associated with reduced packed cell volume (PCV), consistent with previous reports by Kamani et al. (2008) and Shitta (2009). This reduction reflects the pathogenic mechanism of *Babesia spp*., which invade erythrocytes and induce both intravascular and extravascular haemolysis, resulting in anaemia. Consequently, PCV remains a valuable indicator of disease severity in infected dogs.

Although none of the demographic or seasonal variables evaluated showed statistically significant associations with infection, the observed distribution suggests that younger dogs, females, mixed breeds, and dogs sampled during the rainy season may experience greater exposure or susceptibility compared to adults, males, local breeds, and dogs sampled during the dry season, respectively. These patterns should, however, be interpreted with caution, given the absence of statistically significant differences and the small sample size. Similar trends have been reported by Shitta et al. (2012), James-Rugu (2001), Maia et al. (2007), and Arong et al. (2011). The relatively higher occurrence among puppies may reflect immunological immaturity, whereas management practices and reproductive behaviour may contribute to the increased exposure observed in female dogs. Likewise, the greater occurrence during the rainy season is consistent with the favourable environmental conditions that promote tick survival, reproduction, and transmission of Babesia parasites.

An important finding of this study was the superior diagnostic performance of Giemsa- and Field-stained thin blood films for the microscopic detection of canine babesiosis, with both yielding an identical mean parasitaemia of 1.11 after numerical transformation. This diagnostic performance is attributable to their Romanowsky stain composition, which combines acidic (eosin) and basic (azure and methylene blue) dyes to provide excellent contrast between *Babesia* parasites and erythrocytes (Petithory et al., 2005; Barcia, 2007). Babesia organisms therefore appear as sharply defined blue-to-purple intra-erythrocytic bodies against the pale pink background of erythrocytes, allowing accurate visualization of parasite morphology and facilitating reliable estimation of parasitaemia, even when parasite density is relatively low. While Giemsa stain offers superior morphological detail and remains the reference method for microscopic diagnosis, Field stain achieves comparable parasite visualization with a much shorter staining time, making it particularly suitable for rapid diagnosis in routine veterinary practice. Overall, the findings reinforce the continued relevance of stained thin blood film microscopy as a practical, inexpensive, and informative diagnostic approach for canine babesiosis, particularly in resource-constrained veterinary laboratories where assessment of both infection status and parasitaemia is essential for clinical management.

CONCLUSION

Conclusion and Recommendations

Canine babesiosis remains an important tick-borne disease among dogs in Makurdi Metropolis, with an overall prevalence of 13.48%. Although no significant associations were found between infection and sex, breed, age, season, or location, higher infection rates were observed among puppies, female dogs, mixed breeds, and during the rainy season. Infected dogs also had significantly lower packed cell volume values, confirming the haemolytic effects of *Babesia canis* infection. Both Giemsa- and Field-stained thin blood films reliably detected *Babesia* parasites and enabled estimation of parasitaemia. Giemsa stain provided superior morphological detail, whereas Field stain offered comparable diagnostic performance with a shorter staining time, making both methods valuable for the routine diagnosis and clinical management of canine babesiosis.

Routine microscopic screening of dogs using Giemsa- or Field-stained thin blood films, coupled with effective tick control measures and owner education, is recommended to reduce the burden of canine babesiosis. Further studies incorporating larger sample sizes and molecular diagnostic techniques are warranted to strengthen epidemiological surveillance and improve disease detection.

DECLARATIONS

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Authors' Contributions

Nsadzetsen Gilbert. A: Conceptualization, investigation, data collection, data analysis, and drafting of the manuscript.

Nongo Nicholas: Conceptualization, investigation, data collection, data analysis, and drafting of the manuscript, editing, review, and supervision

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Figures

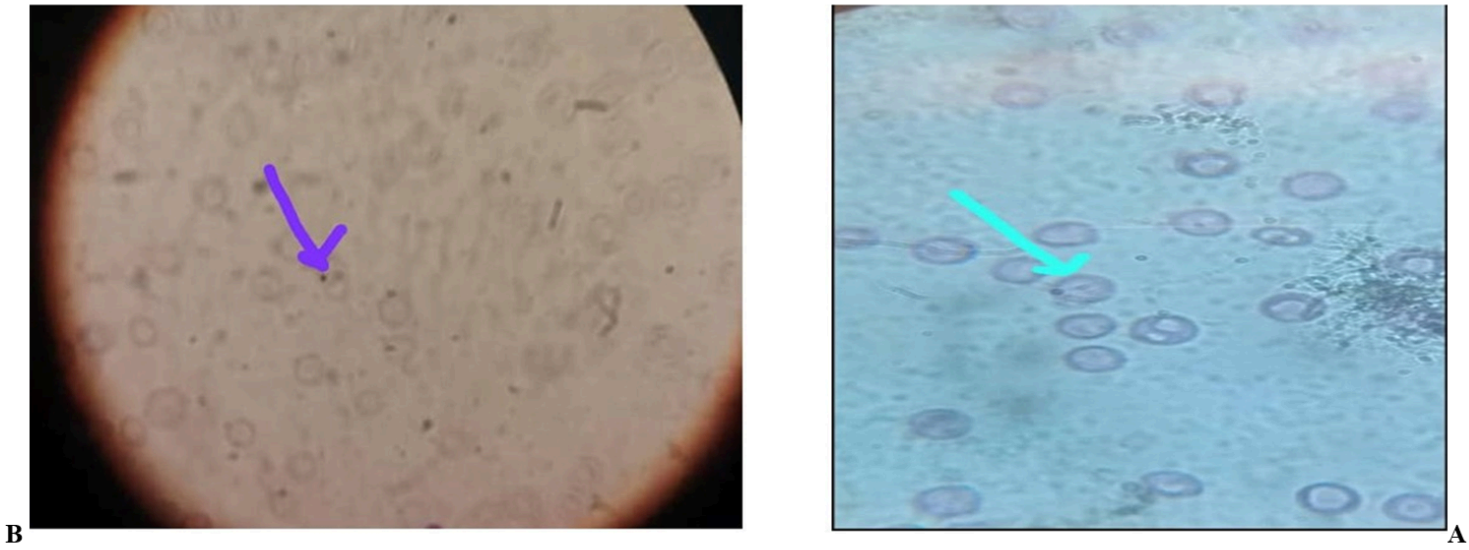


Figure 1

Positive slide of *babesia canis* parasite stained with Field stain (A) and Giemsa stain (B)