



Prevalence of *Babesia Spp.* in Presumably Healthy Dogs and Associated Risk Factors in OBIO/AKPOR Local Government Area, Rivers State, Nigeria

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ABSTRACT

Babesia canis is a parasitic protozoan transmitted by Ixodid ticks. It infects the red blood cells of most mammals especially dogs, causing canine babesiosis. In the present study, the prevalence of *Babesia spp.* and associated risk factors among dogs in Obio/Akpor Local Government Area, Rivers State were investigated using blood film. Blood samples from 150 dogs were randomly collected and examined for the presence of the parasite with March and November, 2022. Blood films were prepared, fixed in methanol, stained in Giemsa and examined under the microscope for the presence of the parasite. Data on age, breed, sex and other related risk factors were obtained using self-structured questionnaire. Out of the 150 dogs examined, 27(18%) were infected and out of the 27 infected dogs, 3(11.1%), 10(37.0%), 2(7.4%), 6(22.2%), 2(7.4%) and 4(14.8%) were from Rumuolumeni, Ogbogoro, Rumuopirikon, Choba, Rumuola and Ozuaba communities respectively. There was a significance difference ($p < 0.05$) in the number of infected dogs across the communities when compared to the number of dogs that were not infected. More males 12(19.4%) were infected than females 6(14.3%). Dogs within the age range of 7-36months had the highest infection 16(59.3%) while no infection was recorded among dogs within the age group of 3-6months. Local breed had the highest infection 18(36%) than crossed breed 6(12%) and exotic breed 3(6%). There was a significance difference ($p < 0.05$) in the prevalence of *Babesia spp.* in relation to sex, agePp and breed of dogs. Other risk factors identified were management practice, vaccination, use of dogs and intensity of tick infestation which were all statistically significance ($p < 0.05$) The study confirmed that canine babesiosis is a serious health concern among dogs in the study area and attention should be given to the risk factors during intervention.

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INTRODUCTION

Dogs are one of the most important domesticated animals in many parts of the globe where they are used for security, hunting and as pets (Omudu *et al.*, 2007). In Africa, dogs are kept for similar purposes including breeding, herding and source of protein (Opera *et al.*, 2005; Hambolu *et al.*, 2014) and for the treatment of certain illness (Gurumyen *et al.*, 2020). Incidentally, dogs are one of the many targets of *Babesia spp.* especially because they are vulnerable to tick infestation (Omudu *et al.*, 2007; Solano-Gallego *et al.*, 2016). The parasites infect a wide range of both domestic and wide animals including man (Carter, 2001; Schnittger *et al.*, 2012).

Babesia canis is a tick-borne parasitic protozoan with global distribution (Otranto *et al.*,

2009). It has been reported in Mexico (Lira-Amaya *et al.*, 2017), Argentina (Mascarelli *et al.*, 2016), Chile (Dicataldo *et al.*, 2020), Peru (Temoche *et al.*, 2018), Collumbia (Galvan *et al.*, 2018), Brazil (de Sousa *et al.*, 2018), Costa Rica (Wei *et al.*, 2015), Poland (Dwuznik-Szarek *et al.*, 2022), United State (Birkenheuer *et al.*, 2004), Europe (Caccio *et al.*, 2002; Beck *et al.*, 2009; Carcy *et al.*, 2015; Rene *et al.*, 2012), Zambia (Nalubam *et al.*, 2015), Tanzania (Bloch *et al.*, 2018), Kenya (Githaka *et al.*, 2022) and Nigeria (Omudu *et al.*, 2007; Jegede *et al.*, 2014; Obeta *et al.*, 2020). The parasite belongs to the genus *Babesia* and, alongside other species of the genus, are responsible for babesiosis in dogs, horses and rodents (Oguche *et al.*, 2020). There are two groups of *Babesia*: the large and small *Babesia*. *Babesia canis* is a large form. They can be morphologically differentiated by

their size and shape in the infected red blood cell (Laha et al., 2015). The large forms with pyriform shape is pointed at one end and round at the other) orientate in the red blood cell in acute angle to each other while the small forms (oval shape lacking pyriform) lies at an obtuse angle to each other (Ruprah, 1985; Laha et al., 2015). Other members of the genus include *Babesia rossi*, *Babesia vogeli*, *Babesia gibsoni* and *Babesia microti* (Jegade et al., 2014; Nalubamba et al., 2015; Rene-Marllet et al., 2015; Obeta et al., 2020). These species, except *Babesia microti* have been reported in Africa (Solano-Gallego and Baneth, 2011).

Babesia canis causes canine babesiosis also known as malignant jaundice (Penzhorn et al., 2017) or piroplasms (Irwin, 2009). The parasite dwells in the red blood cells where it replicates and destroy the erythrocytes causing disease to the host. The commonest mode of spreading the infection is through tick bite during blood meal (Jegade et al., 2014; Nalumanba et al., 2015). Transmission through blood transfusion and transplacental transmission have also been reported (Jegade et al., 2014).

Hard ticks are the major vectors of babesiosis. *Dermacentor riculations* transmits *B. canis* in Europe (Barker et al., 2012), *Rhipicephalus sanguineus* transmits *B. vogel* in tropical and sub-tropical regions of the world (Lavan et al., 2018), including Asia, North America, North and East Africa (Hauschild et al., 1995; Oguche et al., 2020) but in South Africa, *B. rossi*, which causes a fatal in infection in dogs is transmitted by *Haemaphysalis sanguineus* (Bashir et al., 2009; Avenant et al., 2021). The pathological presentation and severity of the infection is dependent on the species of *Babesia* responsible for the infection and the host immune response (De Tommani et al., 2013). However, the general manifestation may include anaemia, lymphopenia, neutropenia and thrombocytopenia (Mathe et al., 2006). Other symptoms include weakness, jaundice, pallor, hypotoxic injury, systemic inflammation fever, splenomegaly and collapse resulting from intra and extra vascular hemolysis (Irwin, 2009; Oguche et al., 2020). In humans, infection by canine babesiosis can results in serious diseases condition especially in immune-compromised persons (Salano-Gallego et al., 2016) but may presents slight symptoms in immune-competent individuals (Vannier and Kraus, 2012; Yabsley and Shock, 2013).

Although several reports of Babesiosis have been documented in Nigeria since its emergence in 1962 (Obeta et al., 2020), scanty record exist on the prevalence of canine babesiosis among dogs in Rivers State particularly in Obio/Akpor Local Government

Area. This study is therefore aimed at the determination of the prevalence and associated risk factors of the infection among dogs in some communities of Obio/Akpor Local Government Area.

MATERIALS AND METHODS

The study was conducted in Obio/Akpor Local Government Area of Rivers State, Nigeria. The Local Government area is located in the Niger Delta and lies along the Bonny River with an estimated population of 3,171,076 inhabitants (Demographia, 2021). It covers an area of about 369Km² and lies on latitude 4^o49'27''N longitude 7^o2'1''E, with an average temperature and humidity of 22^oC and 90% respectively. The LGA is above sea level and it is characterised a tropical climate as in other cities of the Niger Delta. Rainfall is significant most months of the year, and the short dry season has little effect. Six communities within the LGA were randomly selected for this study. The communities were Rumuolumeni (4^o 48' 21'' N, 6^o 56'37''E), Ogbogoro (4^o 52' 27'' N, 7^o 56' 30''E), Rumuepirikom (4^o 54' 4'' N, 6^o 58' 15''E), Rumuola (4^o 50' 7'' N, 7^o 0' 1'' E), Ozuoba (4^o 51' 4'' N, 6^o 58' 51'' E) and Choba (4^o 54' 4'' N, 6^o57'49''E) respectively (Fig. 1).

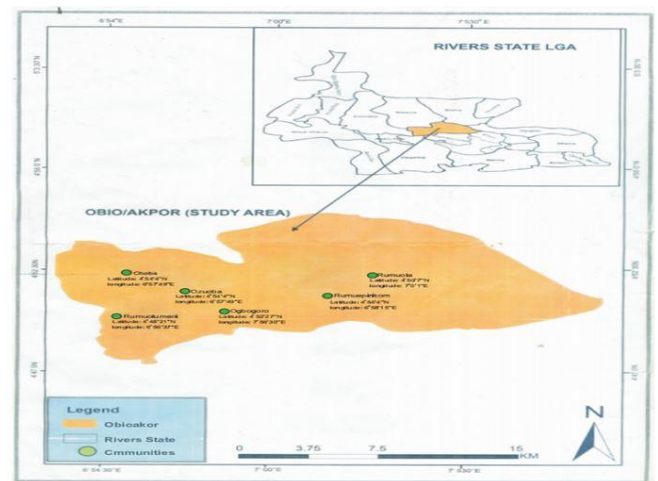


Fig. 1.0: Map of study area

Sample size

The sample size for this study was determined using the method of Kothari (2004).

$$S = \frac{N}{1+N(a)^2}$$

Where:

S = Sample size

N = Population size under the study which is the total number of dog population officially registered by the veterinary unit of Ministry of Agriculture in Obio/Akpor Local Government Area.

a = Level of significance, which is 0.05

$$S = \frac{240}{1+240 (0.05)^2}$$

$$\begin{aligned} &= \frac{240}{1+240(0.0025)^2} \\ &= \frac{240}{1+0.6} \\ &= 150 \end{aligned}$$

Therefore, a total of 150 dogs (25 dogs from each of the communities) were randomly selected for this study. The communities were Rumuolumeni, Ogbogoro, Rumuepirikom, Rumuola, Ozuoba and Choba.

Sample collection

A total of 150 dogs (50 local breed, 50 exotic breed and 50 cross breed) were randomly selected for investigation for the presence of *Babesia canis* infection in the study area. Blood samples from these dogs were obtained the help of a veterinary doctor and the owners. The method of WHO, (1991) which was adopted in the collection of blood samples. About 10ml of blood sample was collected through the cephalic vein using 5mL disposable syringe and 23-gauge needle into a sample vial containing 1mg ethylene diamine tetra acetate-K (EDTA-K) as anticoagulant. The blood samples were immediately kept inside a cool box containing icepack and transported within 4 hours to the Research Laboratory, Department of Biology, Ignatius Ajuru Ignatius Ajuru University of Education, for parasitological examination.

Parasitological analysis of blood samples

Laboratory examination of the blood samples for the presence of *Babesia canis* were done using the method of Hendrix and Robinson (2006). Thin blood smears were prepared from the blood samples, air dried and fixed in methanol for 3-5 minutes and allowed to dry. The slides were stained in 3% Giemsa for 30 minutes and washed with phosphate buffered saline (PBS) to remove excess stains. The slides were then air-dried and examined under oil immersion (x100) for presence of intra- erythrocytic merozoites of *Babesia spp.*

Questionnaires

A total of 150 copies of self-structured questionnaires were produced and distributed to the dog owners to obtain information regarding the sample location, sex, age, breed, management and infestation of ticks as well as the risk factors associated.

Data analysis

The data collected was analyzed using SPSS version 20 to determine the prevalence while Chi-square was used to evaluate the relationship between the variables including age, sex, breed and risk factors. Value of P<0.05 was considered significant and confidence interval of 95%. The following formulae were used to determine the prevalence in relation to the respective variables.

(i) Overall prevalence of *Babesia canis* (OP)

$$= \frac{\text{Total number of dogs infected}}{\text{Total number of dogs examined}} \times 100$$

(ii) Prevalence of *Babesi canis* in relation to sex

$$= \frac{\text{Total number of infected dogs per sex}}{\text{Total number of dogs examined per sex}} \times 100$$

(iii) $\frac{\text{Total number of infected dogs per age}}{\text{Total number of dogs examined per age}} \times 100$

Prevalence of *Babesi canis* infection in relation to age of dogs =

(iv) Prevalence of *Babesi canis* infection in relation to breed of the dogs =

$$\frac{\text{Total number of dogs infected per breed}}{\text{Total number of dogs examined per breed}} \times 100$$

(v) Evaluation of potential risk factors =

$$\frac{\text{Total number of respondents per each potential risk factor}}{\text{Total number of respondents}}$$

Ethical Clearance

The ethical clearance for this study was obtained from the Rivers State Ministry of Agriculture, Port Harcourt, Directorate of Research and Development, Ignatius Ajuru University of Education while verbal consent obtained from dog owners.

RESULTS

Overall Prevalence of *Babesia canis* in dogs

Blood samples from a total of 150 dogs were examined for the presence of *Babesia canis*, out of which 27 (18%) were positive for the parasite. Out of the 27 infected dogs, 3 (11.1%), 10 (37.0%), 2 (7.4%), 6 (22.2%), 2 (7.4%) and 4 (14.8%) were from Rumuolumeni, Ogbogoro, Rumuopirikon, Choba, Rumuola and Ozuaba communities respectively (Table 1). The parasites were identified as pear-shape bodies in red blood cells examined under the microscope (Plate 1).

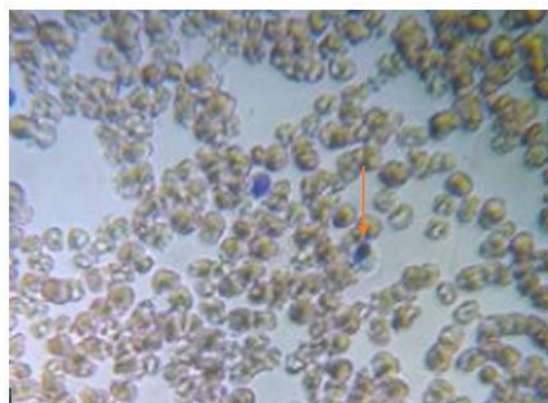


Plate 1: Merozoite (Giemsa Stained) of *Babesia canis* in infected red blood cell

Table 1: Overall prevalence of *Babesia canis* infection among dogs in Obio/Akpor

Locations	No. of dogs Examined	No. Positive (%)	P-Value P<0.05
Rumuolumeni	25	3 (11.1)	0.000002
Ogbogoro	25	10 (37.0)	
Rumuepirikom	25	2(7.4)	
Choba	25	6(22.2)	
Rumuola	25	2(7.4)	
Ozuoba	25	4(14.8)	
Total	150	27(18)	

Prevalence of *Babesia* spp. infection in relation to sex, age and breed of dogs

A total of 150 dogs (108 males and 42 females) were examined. Out of the 108 males and 42 females examined, 21 (19.4%) and 6 (14.3%) were positive for the parasite respectively (Table 2). Similarly, all the 150 dogs investigated were within the age of 3-60months. A total of 42, 71 and 37 dogs were within the age group of 3-6months, 7-36 months and 37-60 months respectively. The results indicates that out of the 42 dogs within the age range of 3-6 month examined, there was no infection 42 (0%), of the 71 dogs within the age range of 7-36 months, 16 (22.5%) were positive while out of the 37 dogs in the age group of 37-60months examined, 11 (29.7%) were infected (Table 2). Out of the 150 dogs examined, 50 each were local, exotic and crossed breeds. Local breed had the highest numerical infection 18 (36%), followed by crossed breed 6 (12%) and exotic breed 3 (6%). There was no significance difference (p>0.05) in the prevalence of the infection in relation to sex, age and breed of dogs investigated.

Table 2: Prevalence of *Babesia canis* infection in relation to sex, age and breed of dogs

Variables	No. examined	No. Infected (%)	p-value
Sex			
Male	108	21 (19.4)	4.60
Female	42	6 (14.3)	
Total	150	27 (18)	
Age (Months)			1.08
3-6	42	0(0)	
7-36	71	16(59.3)	

37-60	37	11(40.7)	
Total	150	27(18)	
Breed			1.96
Local	50	18(36)	
Exotic	50	3(6)	
Crossed	50	6(12)	
Total	150	27(18)	

Prevalence of *Babesia* spp. in relation to risk factors

A total of 150 questionnaires containing self-structured questions were produced and distributed to dog owners with the view to evaluating certain risk factors associated with babesiosis. Out of the 150 respondents, 88 and 62 agreed that they keep stray dogs and caged dogs respectively. Of the 88 stray dogs, 18(66.7%) were infected while 9(33.3%) of the caged dogs were positive for babesiosis (Table 3). Similarly, 21(77.8%) of dogs that had tick infestation and 6(22.2%) of dogs that had no tick were positive for the infection respectively. A total of 54dogs were regularly vaccinated, out of which 2(7.4%) were infected while 25(92.6%) of the 96 dogs that had no regular vaccination were positive for babesiosis (Table 3).

Table 3: Risk factors associated with the transmission of *Babesia canis* (n =150).

Variables	No. of respondents (dog owners)	No. of dogs infected (%)	P-value
Management Method adopted			
Dogs are not confined (Stray dogs)	88	18 (66.7)	0.002
Dogs are confined (Caged dogs)	62	9 (33.3)	
Total	150	27 (18)	
Tick infestation			
Presence of tick	56	21 (77.8)	0.002
Absence of tick	94	6 (22.2)	
Total	150	27 (18)	
Vaccination			
Regular	54	2 (7.4)	0.04
Not regular	96	25 (92.6)	
Total	150	27 (18)	
Reason for keeping dog			
Pet	36	2 (7.4)	0.02
Hunting	44	16 (59.3)	
Security	70	9 (33.3)	
Total	150	27 (18)	
Area			
Rural	75	19 (70.4)	0.013
urban	75	8(29.6)	
Total	150	27(18)	
Diet			
Pet food	47	7(26.0)	0.006
Home-made food	64	10(37.0)	

Anything	39	10(37.0)
Total	150	27(18)

Out of the 36, 44 and 70 dogs kept as pet, hunter and security respectively, 7(26%), 10(37%) and 10(37%) were positive for babesiosis respectively (Table 3). More rural dogs (70.4%) were infected than urban dogs (29.6%). The infection rate of dogs that were feed on home-made food was 37%, dogs that ate anything had 37% infection rate while dogs that were fed on pet food had 26% (Table 3). The results indicated that these factors significantly ($p<0.05$) influence the prevalence of the infection.

DISCUSSION

Babesia canis is a haemoparasite that causes canine babesiosis in dogs. The infection is highly pathogenic and is the major cause of haemolytic anaemia in dogs in the tropics (Kamani *et al.*, 2011). The parasite is among the most widely distributed haemoparasites of dogs occurring in almost anywhere the tick vector *Rhipicephalus sanguineus* is reported (Taylor *et al.*, 2007).

This study recorded a high overall prevalence of 27% of *Babesia canis* in the study area. This is an indication that babesiosis is still a health challenge in the area and that the tick-vector of the parasite is widely distributed in Nigeria. The recorded prevalence is higher than the 8.9%, 11.66% and 10.8% reported in Abuja at various times by Jegede *et al.*, 2014, Obete *et al.*, 2009 and Obeta *et al.*, 2020 respectively. It is also higher than the 10.2% and 12.9% recorded in Makurdi and Plateau State by Amuta *et al.* (2010) and Oguche *et al.* (2020) respectively; as well as the 2.4%, 3.8%, 5.3% and 13.33% in Zambia, Cape Verde, Souther France and Costa Rica by Williams *et al.* (2014), Salem and Farag (2014), Garcia-Quesada *et al.* (2021) and Rene-Martellet *et al.* (2015) respectively. The differences in the prevalence recorded in the various studies may be attributed to the poor management strategies adopted by the dog owners (Obeta *et al.*, 2020), relative distribution and abundance of the tick-vector of the parasite, differences in geography of study areas (Jegede *et al.*, 2014) and lack of immunization of the dogs by their owners (Amuta *et al.*, 2010).

In this study, more males were infected than females and this was statistically significant ($p<0.05$) (p -value=?). This is in consonance with previous studies in Jos, Nigeria by Omudu *et al.* (2007), in Vom, Northern Nigeria by Daniel *et al.* (2016) and elsewhere in South Africa by Mellanby *et al.* (2011). The high prevalence could be as a result of the hormonal status of the male dogs particularly the presence of testosterone which might limit the quality of care given

to it by the owner, excessive roaming behaviours of male dogs to search for mating partners and establish territories, exposing them to more tick infestation (Mellanby *et al.*, 2011, Daniel *et al.*, 2016; Obeta *et al.*, 2020). Their female counterparts are presumably less mobile as they spend much time nursing the puppies and are giving good care and attention by the owners due to their economic value. The result is however contrary to the records of Omudu *et al.* (2010) in Makurdi, Okunbanjo *et al.* (2013) in Abuja, Jegede *et al.* (2014) in Abuja and Oguche *et al.* (2020) in Jos. These studies recorded high prevalence of *B. canis* in female more than male dogs.

The study recorded a high infection rate ($p<0.05$) (p -value=?) of *Babesia* spp. in older dogs while there was no observable infection in younger dogs. Specifically, dogs within the age range of 7-36months had the highest infection. This is in agreement with previous studies by Obeta *et al.* (2020) and Jegede *et al.* (2014) who reported a high prevalence of the parasite in older dogs in Abuja. Similar observation was made in Jos by Oguche *et al.* (2020). This result contradicts the report of Okunbanjo *et al.* (2013) who observed a low prevalence of the parasite in older dogs and a high prevalence in puppies in Zaria. The relatively high prevalent rate recorded in this study could be attributed to lower resistant and poor immune system against the parasite by older dogs, possibly because of age. Research indicates that animal immunity decreases with age making them susceptible to infection. It may also be attributed to frequent and longtime exposure of older dogs to the vector of the parasite (Egege *et al.*, 2009). It is posited that dogs within the age range recorded in this study are very active and roam about indiscriminately thereby exposing themselves to tick infection which might account for the high prevalent rate of canis babesiosis recorded in our study. Again, the habit of assembling in the mating season and style of playing in the field may influence high tick infestation of dogs. However, studies have shown that canis babesiosis increases with age but declines when the dogs are about 4-5years old (Hornok *et al.*, 2006).

In this study, local breed has the highest infection ($p<0.05$) of 36% compared to exotic breed (6%) and crossed breed (12%). Similar result was recorded in Abuja by Jegede *et al.* (2014) and Daniel *et al.* (2006) in Jos.

This could be as a result of poor management system and lack of care for the local breed by their owners. This local breed is allowed to roam freely in the street to scavenge, hence, they are vulnerable to high tick infestation. Moreso, they are hardly immunized and are breed in very poor hygienic

condition (Kamani *et al.*, 2011; Eguche *et al.*, 2020). In this study, there was no recorded significant prevalence in babesiosis infection in relation to breed of dogs. However, several studies have suggested that breed is a predisposing risk factor in babesiosis infection (Hornk *et al.*, 2006; Mellanby *et al.*, 2011). The reason for this is not clear but may be related to differences in genetic composition (Obeta *et al.*, 2020). The result obtained in our study is at variant with the report of Nalubamba *et al.* (2015) and Obeta *et al.* (2020). The researchers reported high prevalence of canis babesiosis in exotic breed in Zambia and Abuja, Nigeria respectively.

Unconfined dogs, presence of tick on dogs, irregular vaccination were the risk factors associated were some of the risk factors identified. Dogs used for hunting had high frequency of infection than others. This is in agreement with previous studies (Costa-junior *et al.*, 2009; Veneziano *et al.*, 2018). This might be as attributed to the adopted management techniques. The results also indicated that dogs in urban and rural areas are equally vulnerable to the infection. This is in consonance with the record of Silva *et al.* (2012).

CONCLUSION

Babesia spp. remains a health challenge among dogs in the study area irrespective of the risk factors. However, free-range and hunting dogs were at high risk of infection. Due to the physiological, behavioural and nutritional effect of the canis babesiosis on dogs, prevention and control remains a viable option, through modern management system, vaccination, use of acaricide and regular fumigation of the environment.

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Conflict of interest

The authors declared that there is no conflict of interest.

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