

Review

An annotated checklist of tick-borne pathogens of dogs in Nigeria

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ABSTRACT

Tick-borne pathogens are transmitted by the bite of a tick vector or the ingestion therein by a susceptible host. Conventionally, the diagnosis of tick-borne parasites in Nigeria is achieved through the demonstration of their developmental stages in the host's blood or tissues. These classical methods are limited in their capability to detect chronic cases or healthy carriers and to differentiate between pathogens of similar morphological features. Molecular diagnostic techniques of high sensitivity have been developed to overcome these shortfalls by detecting the pathogen's DNA, elucidating their genetic makeup and comparing their phylogenetic relationship to other pathogens, sometimes necessitating taxonomic changes.

Detection of DNA of canine tick-borne pathogens (CTBPs) such as *Ehrlichia canis*, *Hepatozoon canis*, and *Mycoplasma haemocanis* has been reported in Nigerian dogs corroborating earlier microscopic findings. Furthermore, DNA of *Anaplasma platys*, *Ehrlichia ewingii*, 'Candidatus Mycoplasma haemobos' and *Theileria* sp. hitherto considered to be absent in Nigeria has been detected, thereby expanding the spectrum of CTBPs in the country. However, in some cases, diagnoses based on the detection of single short DNA fragments from pathogens can cause considerable confusion. This occurred in the case of the large canine *Babesia*, previously referred to as *Babesia canis* which has been reported in Nigerian dogs. There appears to be confusion regarding the accurate status of this pathogen in Nigeria due to the subsequent classification into *Babesia canis*, *Babesia vogeli* and *Babesia rossi*. Moreover, several molecular studies failed to detect the DNA of *Babesia gibsoni* previously reported in Nigerian dogs by light microscope. These findings necessitate regular update of the list of CTBPs of veterinary and medical importance, in order to keep practitioners abreast with these changes. Herein, an annotated checklist of tick-borne pathogens of dogs in Nigeria, based on available data from both classical and molecular studies, with notes explaining any discrepancies between the methods and probable explanations thereof, is presented.

1. Introduction

Tick-borne pathogens and their consequences on the wellbeing of dogs in Nigeria have been reported since the 1970s (Oduye and Dipeolu, 1976; Aliu et al., 1976; Idowu et al., 1977). These reports were based mainly on the detection of the pathogens in the host's blood and tissues using classical methods until recently when molecular methods were employed (Sasaki et al., 2007; Kamani et al., 2010, 2013a, 2013b; Adamu et al., 2014; Happi et al., 2018). Results from the molecular studies, however not only confirmed the presence of some of the earlier reported pathogens but also detected some species, such as, *Anaplasma platys* and 'Candidatus Mycoplasma haemobos' hitherto considered absent in Nigeria (Kamani et al., 2013a; Happi et al., 2018). Furthermore, DNA attributed to some pathogens such as *Theileria equi*, *Theileria* sp. (sable), and *Ehrlichia ruminantium* generally known to exhibit a high

degree of host specificity, and therefore considered not to infect dogs, were amplified from blood samples of dogs from Nigeria (Adamu et al., 2014). On the other hand, molecular methods failed to detect other pathogens such as *Babesia gibsoni* (Sasaki et al., 2007; Kamani et al., 2010, 2013a; Adamu et al., 2014; Happi et al., 2018) described earlier by light microscopy in Nigerian dogs (Aliu et al., 1976; Ezeokoli et al., 1983). Although the molecular methods are highly sensitive and results generated appear to be reliable, the cost implication precludes their general use in most developing countries like Nigeria. Hence, classical methods are routinely used for most diagnostic purposes, being complemented in some instances with molecular techniques for research investigations. The concurrent use of classical and molecular methods, where indicated, seems to be the optimal diagnostic option. By molecular methods the list of canine tick-borne pathogens (CTBPs) in Nigeria is likely to increase. However, caution should be exercised in the

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interpretation and drawing of conclusions based on preliminary results obtained solely by molecular methods (Uilenberg et al., 2018). One way to guide the research community in this matter is to compile up to date information on the diversity of CTBPs in Nigeria, which is the aim of this article.

2. Materials and methods

A checklist of tick-borne pathogens of dogs in Nigeria was compiled based on the examination of published literature for the years 1970–2018. To ensure the broadest possible coverage of scientific literature, searches were conducted in the following online databases: PubMed, CabDirect, Web of Science (WoS) and African Journals Online (AJOL), using the following keywords; blood, parasites, dog, tick-borne and Nigeria.

A list of pathogens was retrieved following careful evaluation of the information and the diagnostic methods applied. In addition, explanatory notes highlighting key points for each pathogen listed were provided.

3. Checklist of tick-borne pathogens of dogs in Nigeria

Several tick-borne pathogens of dogs have been reported in Nigeria. Their presence has been demonstrated either through the detection of developmental stage(s) in the blood of dogs by light microscopy or by the detection of their DNA using PCR and sequencing methods (Table 1).

Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Family: Anaplasmataceae
Genus: *Ehrlichia*

Ehrlichia canis

Ehrlichia canis, the agent of canine monocytic ehrlichiosis was first identified in dogs in Algeria (Donatien and Lestoquard, 1935). The organism (Fig. 1) has special tropism for monocytes and macrophages, appearing in the form of morulae within the cytoplasm of the latter cells (Huxsoll et al., 1970). Canine monocytic ehrlichiosis is a multisystemic disease manifesting itself in acute, subclinical or chronic forms. The clinical signs vary greatly, ranging from silent infection to severe clinical disease, characterized by depression, lethargy, anorexia, fever, lymphadenomegaly, splenomegaly and hemorrhages such as petechiae, ecchymoses and epistaxis. *Ehrlichia canis* is primarily a pathogen of dogs and despite some preliminary molecular reports suggesting that a closely related organism infects humans in Venezuela and Costa Rica (Perez et al., 1996, 2006; Bouza-Mora et al., 2017), it is not considered to cause disease in humans.

Status in Nigeria

The presence of *E. canis* has been demonstrated in the blood of dogs in Nigeria by both microscopic detection of morulae and the molecular amplification of the DNA targeting the 16S rRNA gene of the organism (Ezeokoli, 1978; Ezeokoli et al., 1983; Kamani et al., 2011, 2013a,b; Adamu et al., 2014; Happi et al., 2018). Furthermore, molecular or serological evidence of *E. canis* has been reported in many other African countries such as Cameroon, Tunisia, Senegal, Chad, South Africa, Egypt and Zimbabwe (Brouqui et al., 1991; Ndip et al., 2005).

Ehrlichia ewingii

Ehrlichia ewingii infects canine granulocytic cells and is the agent of a canine granulocytic ehrlichiosis (Anderson et al., 1991, 1992). It is a chronic condition characterized by polyarthritis in infected dogs (Anderson et al., 1992). Abnormal hematological findings include leukocytosis, lymphopenia, thrombocytopenia, hyperglobulinemia, bone marrow hypoplasia, and plasmacytosis of parenchymal tissues (Ewing et al., 1971; Bellah et al., 1986; Cowell et al., 1988; Anderson et al., 1992). *Ehrlichia ewingii* is the agent of human granulocytic ehrlichiosis manifested by fever, myalgia, headache and malaise in humans (Buller

et al., 1999).

Status in Nigeria

Ehrlichia ewingii was detected by light microscopy as a co-infecting agent with other tick-borne pathogens in dogs in Ibadan Nigeria (Happi and Anita, 2012). This was the first and only report of this pathogen in dogs from Nigeria. Although not each morula of *Ehrlichia* (or *Anaplasma*) seen in granulocytes is necessarily *E. ewingii*, it should be noted that it could also be *A. phagocytophilum*. Thus, molecular confirmation (by PCR and sequencing) is required to validate this finding, considering limitations of the method used as well as epidemiological factors involved in the transmission of this pathogen. Interestingly, DNA of *E. ewingii* has been reported in dogs from Cameroon (Ndip et al., 2005), a neighboring country to Nigeria, suggesting that the detected organism was indeed *E. ewingii*.

Genus: Anaplasma

Anaplasma platys

Anaplasma platys (formerly, *Ehrlichia platys*) was first described in dogs from Florida, United States of America (Harvey et al., 1978). It is an obligatory intracellular bacterium that infects platelets (Fig. 2) and causes canine infectious cyclic thrombocytopenia. *Anaplasma platys* infection was originally considered to be subclinical (Harvey et al., 1978). However, there are reports of more virulent strains of this agent in several countries of the Mediterranean basin suggesting it can cause signs similar to those caused by *E. canis* however with lower severity. The disease is characterized by cyclic thrombocytopenia (Walker et al., 1970; Harrus et al., 1997; Aguirre et al., 2006). Although a single report associated *A. platys* with a disease in 2 women from Venezuela (Arraga-Alvarado et al., 2014), *A. platys* is not considered to be pathogenic to humans.

Status in Nigeria

There are a few reports of *A. platys* infection in dogs from Nigeria. Happi and Anita, (2012) using light microscopy, detected *A. platys* in co-infection with *Hepatozoon canis* in one dog. Additionally, the DNA of *A. platys* was detected in Nigerian dogs (Kamani et al., 2013a). These findings suggest that the bacterium might have been long infecting dogs in Nigeria, but has been overlooked or misdiagnosed during microscopic examination of blood smears. Microscopists and diagnosticians in Nigeria should pay particular attention to typical *A. platys* inclusions in the form of morulae within platelets during evaluation of blood smears for the presence of haemoparasites. Preparation and examination of buffy coat smears may be invaluable for the detection of *A. platys* infections using light microscopy.

Phylum: Tenericutes
Class: Mollicutes
Order: Mycoplasmatales
Family: Mycoplasmataceae
Genus: *Mycoplasma*

Mycoplasma haemocanis

Mycoplasma haemocanis (formerly *Haemobartonella canis* or *Eperythrozoon canis*) is a canine hemotropic mycoplasma (haemoplasma), first observed by Kikuth (1928). It is a wall-less epi-erythrocytic rod or coccoid bacterium causing haemolytic anemia in dogs. To date, haemoplasmas have not been cultured (Sykes et al., 2008). The bigger forms of the organism can be observed in peripheral blood smears by light microscopy while their small species e.g. '*Candidatus Mycoplasma turicensis*', that is known to infect cats, cannot be visualized microscopically (Messick, 2004). Immunocompetent dogs are usually presented with subclinical infection but immunocompromised or splenectomized dogs may exhibit signs of anemia, loss of weight, fever, anorexia and lethargy (Messick et al., 2002). Until now, there is no evidence that canine haemoplasmas cause human disease, although human eperythrozoonosis has been reported in Europe, North America and eastern Asia (China), perhaps associated with *Eperythrozoon suis* from pigs or novel haemoplasmas (Yang et al., 2007; Hu et al., 2009; Yuan et al., 2009; Steer et al., 2011).

Status in Nigeria

Table 1
Tick-borne diseases of dogs reported in Nigeria by light microscopy and molecular techniques.

Disease	Etiology	Proven/putative vector	Distribution	Reported prevalence (%)		References
				Microscopy	Molecular	
Canine piroplasmosis	<i>Babesia canis</i>	<i>Dermacentor reticulatus</i>	Europe	3.5, 4.1, 8.9, 9.3, 10.2, 11, 26	65.5, a single case report	Oduye and Dipeolu, 1976; Idowu et al., 1977; Ezeokoli et al., 1983; Omudu et al., 2007; Okubanjo et al., 2013; Jegede et al., 2014; Kamani et al., 2010; Happi et al., 2018, Kamani et al., 2013a; Adamu et al., 2014
	<i>Babesia vogeli</i>	<i>Rhipicephalus sanguineus</i> (s.l.)	Africa, Asia, Europe, North/Central/South America, Australia	None	0.6, 1	Sasaki et al., 2007; Kamani et al., 2013a; Adamu et al., 2014
	<i>Babesia rossi</i>	<i>Haemaphysalis elliptica</i>	Sub-Saharan Africa	None	6.6, 11, 38	Sasaki et al., 2007; Kamani et al., 2013a; Adamu et al., 2014
	<i>Babesia gibsoni</i>	<i>H. longicornis</i> , <i>H. hystricis</i> , <i>H. hispinosa</i> ?, <i>R. sanguineus</i> (s.l.)?	Southeast Asia, United States, South America, Australia, Europe	0.8, 20.2	None	Oduye and Dipeolu, 1976; Idowu et al., 1977
	<i>B. microti</i> -like (<i>Theileria annae</i>)	<i>Ixodes hexagonus</i>	Europe, North America, Asia	None	0.6	Kamani et al., 2013a
	<i>Theileria equi</i>	Ticks of the genera <i>Rhipicephalus</i> , <i>Hyalomma</i> and <i>Dermacentor</i>	Africa, Europe, Asia, Central and South America.	None	3	Adamu et al., 2014
Canine monocytic ehrlichiosis	<i>Ehrlichia canis</i>	<i>R. sanguineus</i> (s.l.)	Global	5.1, 10.3	5, 11, 36.2, 46.1	Ezeokoli et al., 1983; Kamani et al., 2013a, 2013b, Adamu et al., 2014; Happi et al., 2018
Canine granulocytic ehrlichiosis	<i>Ehrlichia ewingii</i>	<i>Amblyomma americanum</i>	Global	3/8 case report	None	Happi and Anita, 2012
Canine infectious cyclic thrombocytopenia.	<i>Anaplasma platys</i> (formerly, <i>Ehrlichia platys</i>)	<i>R. sanguineus</i> (s.l.), <i>D. auratus</i> , <i>Hyalomma truncatum</i>	Worldwide	1/8 case report	6.6	Happi and Anita, 2012, Kamani et al., 2013a
Canine hemotropic mycoplasma	<i>Mycoplasma haemocanis</i>	Unknown, but thought to occur via arthropods	Tropical and subtropical regions of the world	8.6	13.3, 17.9	Oduye and Dipeolu, 1976
Hepatozoonosis	<i>Hepatozoon canis</i>	<i>R. sanguineus</i> (s.l.), <i>R. turanicus</i> , <i>R. appendiculatus</i> , <i>R. simus</i> , <i>H. longicornis</i> , <i>H. flava</i> , <i>A. ovale</i> , <i>A. hebraicum</i> , <i>A. marmoreum</i>	Africa, Asia, Europe, South and Central America	10.7, 22, 30.2	41.4,	Aquino et al., 2016, Happi et al., 2018 Ezeokoli et al., 1983; Kamani et al., 2013a, Okubanjo et al., 2013; Opara et al., 2017
Filaroids	<i>Acanthocheilonema dracunculoides</i>	<i>Hippobosca longipennis</i> , <i>R. sanguineus</i> (s.l.)	Africa, Asia and Europe	Not available	Nil	Schillhorn van Veen, 1974

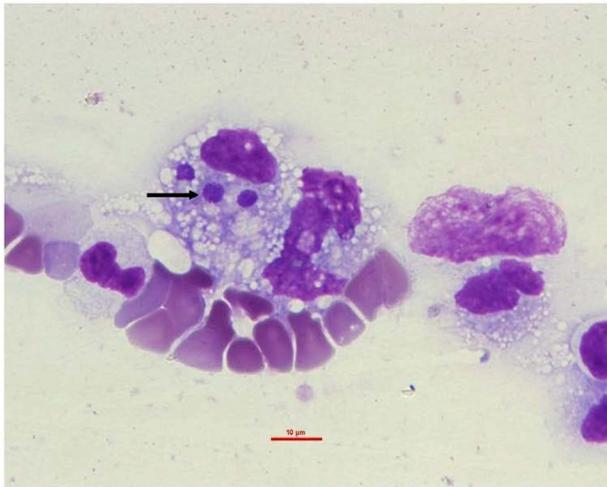


Fig. 1. Giemsa stained blood smear from a naturally infected dog showing 3 *Ehrlichia canis* morullae (arrow) in an activated monocyte. x100 objective.

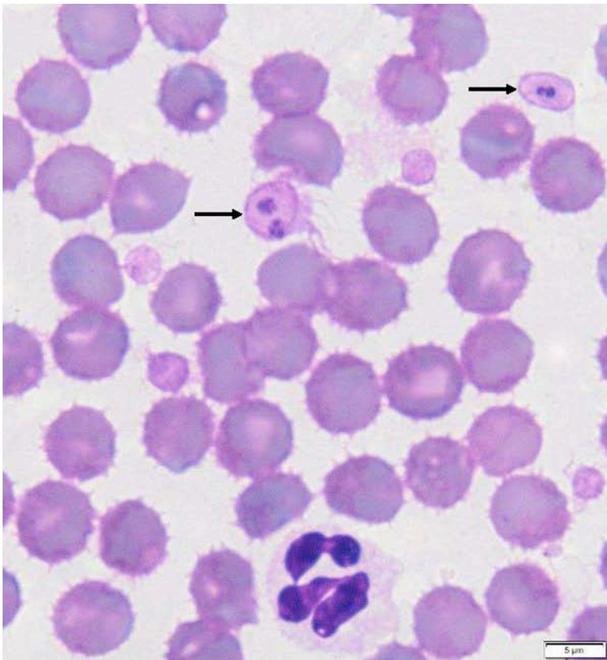


Fig. 2. Blood smear from a dog showing platelets containing *Anaplasma platys* morullae (arrows). Giemsa stain x100 objective.

Mycoplasma haemocanis (named in the original article as *Haemobartonella canis*) infection was reported in 43 out of 500 dogs from Nigeria, screened for haemoparasites by light microscopic examination of Giemsa stained blood smears (Oduye and Dipeolu, 1976). The same authors reported *Eperythrozoon* spp. as a different pathogen infecting the examined dogs. In another study, DNA of haemoplasmas was detected in 44 of 245 (17.9%) dogs from Nigeria using a generic haemoplasma qPCR assay. Nucleotide sequences from the study showed that the Nigerian dogs were infected with *M. haemocanis* (Mhc) and ‘*Candidatus* *Mycoplasma haematoparvum*’ (CMhp) (Aquino et al., 2016). Recently, Happi et al. (2018) reported the detection of *M. haemocanis* and a cattle-related species ‘*Candidatus* *Mycoplasma haemobos*’ in dogs from Nigeria using PCR and sequencing methods. More work is required to establish if the cattle-related species ‘*Candidatus* *Mycoplasma haemobos*’ indeed infects dogs in Nigeria or if it is an accidental finding due to erroneous amplifications or laboratory contamination sometimes associated with sensitive technique like the PCR.

Phylum Apicomplexa
Class: Piroplasmata/Aconoidasida
Order: Piroplasmida
Family: Babesiidae
Genus: *Babesia*

Babesia

The first record of canine *Babesia* infection was made in Italy in 1895 not long after the detection of the bovine disease (Roncalli, 2001). Canine babesiosis is caused by intraerythrocytic protozoan parasites of the genus *Babesia* and it is one of the most important tick-borne infectious diseases among dogs. Historically, the classification of the various babesias that infect domestic animals was mainly based on their size, morphology and elements in their life cycles (Mehlhorn and Schein, 1987; Telford and Spielman, 1999).

Previously, all large forms (2.5–5.0 µm) of *Babesia* in dogs were designated *Babesia canis*, whereas all small forms (1.0–2.5 µm) were considered to be *Babesia gibsoni* (Boozar and Macintire, 2003). However, advances in molecular diagnostic methods have also demonstrated that other *Babesia* species such as *Babesia conradae*, *Babesia microti*-like, *Theileria annae*, *Theileria* spp. and a yet unnamed large form *Babesia* sp. infect dogs and cause distinct diseases (Birkenheuer et al., 2004; Irwin, 2009). Among the large babesias; *Babesia rossi*, *B. canis* and *Babesia vogeli*, previously considered as subspecies of *B. canis* (Uilenberg et al., 1989; Hauschild and Schein, 1996), are identical morphologically but have been shown to differ in the severity of clinical manifestations, vector specificity, genetic characteristics, and geographic distributions. They are currently considered as separate species and can be separated by molecular methods (Zahler et al., 1998; Irwin, 2009; Solano-Gallego and Baneth, 2011; Dantas-Torres et al., 2017). Similarly there are at least 3 genetically distinct small piroplasms which infect dogs (Kjemtrup et al., 2000).

Large Babesiae

Of the four large *Babesia* species infecting dogs globally, two have been reliably confirmed in Nigeria.

Babesia rossi

Babesia rossi usually infects both young and adult dogs and is considered to cause the most severe disease manifestations among the large *Babesia* species that infect dogs (Reyers et al., 1998; Jacobson, 2006). A protein named *B. rossi* erythrocyte membrane antigen1 (BrEMA1) is suspected as a virulence factor in *B. rossi* canine babesiosis and is associated with its pathogenicity (Matjila et al., 2009). *Babesia rossi* in blood smears measures approx 2x5µm and usually appears as paired merozoites in the RBC (Oyamada et al., 2005; Sasaki et al., 2007; Matjila et al., 2008). Uncomplicated infection due to *B. rossi* manifests in fever, lethargy, anorexia, pale mucous membranes and splenomegaly. There is mild to moderate anemia, thrombocytopenia, leukocytosis, pigmenturia, bilirubinemia and bilirubinuria. Complicated cases however, present with acute renal failure, icterus, hypotension, acute respiratory distress syndrome (ARDS), vomiting, diarrhoea, pancreatitis, myalgia, rhabdomyolysis, ascites, pulmonary edema, cerebral and renal involvement, peracute manifestations and shock. In addition, metabolic and respiratory acid–base imbalances, renal azotemia, coagulopathy, immune-mediated hemolytic anemia, hypoglycemia and hyperlactemia are commonly observed in complicated cases (Leisewitz et al., 2001; Keller et al., 2004; Jacobson, 2006).

Status in Nigeria

The presence of this pathogen in Nigeria has been demonstrated through PCR amplification of DNA and sequencing of the 18S rRNA gene from blood samples of dogs in Nigeria (Sasaki et al., 2007; Kamani et al., 2010, 2013a; Adamu et al., 2014).

Babesia canis

Babesia canis causes a mild to severe disease in dogs (Uilenberg et al., 1989). *Babesia canis* usually occurs in infected erythrocytes as paired pear or tear-shaped bodies each measuring approx 2 × 5 µm (Bourdoiseau, 2006; Cassini et al., 2009; Iori et al., 2010). Infected dogs present with fever, lethargy, anorexia, dehydration and jaundice. Some

clinico-pathological changes associated with *B. canis* include; mild to moderate normocytic normochromic non regenerative anemia, thrombocytopenia, neutropenia, pigmenturia, bilirubinemia and hemolytic bilirubinuria (Bourdoiseau, 2006; Solano-Gallego et al., 2008; Carli et al., 2009).

Status in Nigeria

Although epidemiological considerations does not favor the occurrence of *B. canis* in Nigeria, there appears to be confusion in the literature in this regard. Since most of the reports were based on light microscopic detection which lacks the capacity to differentiate between the large canine babesias, the findings were simply referred to as *B. canis* in favor of the old nomenclature. However, there are few reports of molecular detection of *B. canis* DNA in Nigerian dogs (Kamani et al., 2010; Happi et al., 2018). Therefore, there is need for in-depth and large scale studies using both classical and molecular methods to validate these findings and to rule out the possibility of erroneous amplification or contamination.

Babesia vogeli

Babesia vogeli usually causes a subclinical to mild or moderate clinical disease in adult dogs (Uilenberg et al., 1989; Carret et al., 1999), but severe to fatal haemolytic anemia has been reported in young dogs and pups (Solano-Gallego et al., 2008). *Babesia vogeli* merozoites measure about $2.5 \times 4.5 \mu\text{m}$ and usually occur singly or in pairs (Fig. 3) in the infected erythrocytes (Jefferies et al., 2003; Oyamada et al., 2005; Passos et al., 2005; Sasaki et al., 2007; Criado-Fornelio et al., 2007; M'Ghirbi and Bouattour, 2008). Fever, lethargy and anorexia are the common clinical signs. None of the large *Babesia* spp. that affects dogs is considered to be of zoonotic importance (Homer et al., 2000).

Status in Nigeria

The presence of *B. vogeli* has been confirmed in Nigerian dogs through the amplification of the parasite DNA by PCR and sequencing of the 18S rRNA gene (Sasaki et al., 2007; Kamani et al., 2013a; Adamu et al., 2014).

Small piroplasms

Small *Babesia* spp. such as; *B. gibsoni*, *B. conradae*, *B. microti*-like piroplasm) have been reported in dogs in several regions of the world (Jefferies et al., 2003; Birkenheuer et al., 2005; Miyama et al., 2005; Kjemtrup et al., 2006; Trapp et al., 2006; Hartelt et al., 2007; Jefferies et al., 2007; Lee et al., 2009). Similarly, short DNA sequences of *Theileria* spp. (*T. equi*, *T. annulata*) have been detected in dog blood samples in several countries (Camacho et al., 2003; Matjila et al., 2008; Beck et al., 2009; Gimenez et al., 2009; Yeagley et al., 2009; Iori et al., 2010; Kamani et al., 2013a; Adamu et al., 2014).

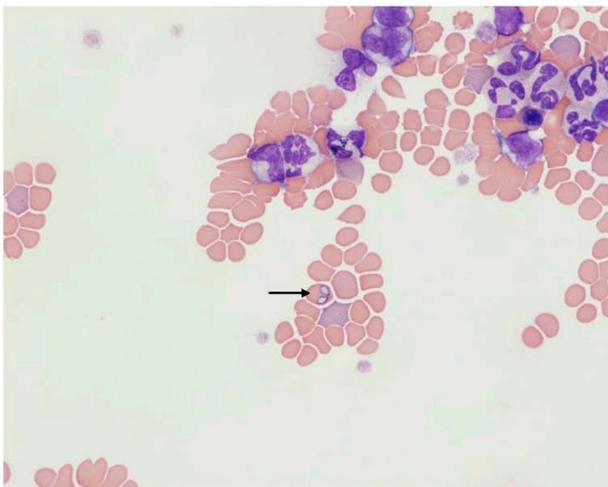


Fig. 3. Blood smear from a dog showing a large *Babesia* spp. (*B. vogeli*) confirmed by PCR and sequencing. Giemsa stain x100 objective.

Babesia gibsoni

Babesia gibsoni was first described by Patton (1910) as a pathogen occurring in dogs in India. This pathogen may cause acute disease or chronic disease that is associated with weight loss and weakness (Birkenheuer et al., 2005). *Babesia gibsoni* merozoites measure approximately $1 \times 3 \mu\text{m}$ and usually occurs singly in erythrocytes. Clinical disease in dogs is characterized by fever, lethargy, pale mucous membranes, jaundice, lymphadenomegaly, splenomegaly and weight loss. Pathological changes in infected dogs include hemolytic regenerative anemia, immune mediated anemia, thrombocytopenia, pigmenturia, bilirubinemia and bilirubinuria (Birkenheuer et al., 1999; Macintire et al., 2002; Meinkoth et al., 2002; Lee et al., 2009). Although ixodid ticks are the proven vectors (Table 1), other reported methods of transmission include, transfusion of infected blood (Stegeman et al., 2003), bites from infected dogs, ingestion of infected blood, the use of contaminated surgical equipment and transplacental transmission (Birkenheuer et al., 2005; Fukumoto et al., 2005; Jefferies et al., 2007; Yeagley et al., 2009), partly accounting presences of the infection in region where its putative vectors are absent.

Status in Nigeria

Until recently, identification of canine *Babesia* in Nigeria was based solely on the morphological appearance of merozoites in blood smears. Hence the traditional classification based on size and appearance of the merozoites as *B. canis* for large and *B. gibsoni* for small piroplasm predominates in literature. Accordingly, *B. gibsoni* was supposedly detected in blood smears of dogs in Nigeria (Idowu et al., 1977; Ezeokoli et al., 1983). However, there is no single report of the detection of *B. gibsoni* DNA in dogs in Nigeria by molecular methods to date (Sasaki et al., 2007; Kamani et al., 2010, 2013a; Adamu et al., 2014; Happi et al., 2018).

Babesia microti-like piroplasm (*Theileria annae*)

This is a small parasite belonging to the *Babesia microti*-complex that was initially detected in the blood of a dog from Spain suffering from clinical babesiosis (Camacho-Garcia, 2006). It appears as small ring-shaped single bodies of $1-2 \mu\text{m}$ per erythrocyte. It is apparently a very common infection in red foxes in some parts of the world. Clinically, the infection is characterized by apathy, fever, severe regenerative anemia, thrombocytopenia, hepatosplenomegaly, haemoglobinuria, azotemia and orangery feces in few cases (Camacho-Garcia, 2006; Miró et al., 2015).

Status in Nigeria

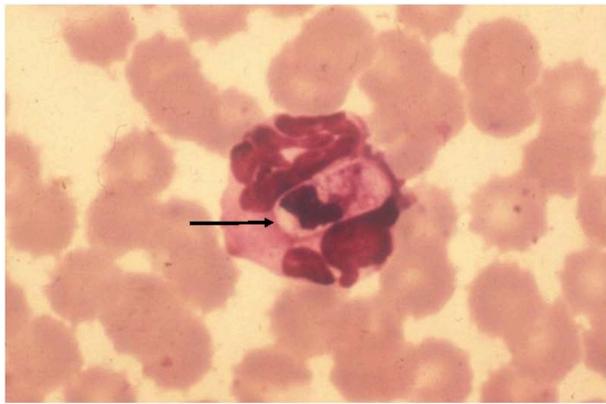
Theileria-like spp. have been reported by molecular amplification and sequencing of the 18S rRNA gene in dogs in Nigeria (Kamani et al., 2013a; Adamu et al., 2014). Elsewhere, other *Theileria* species have been reported in dogs. For example, *T. annulata* was detected from an asymptomatic dog (Criado et al., 2006) whereas *T. equi* was detected from three asymptomatic dogs and one symptomatic dog (Criado-Fornelio et al., 2003) using molecular techniques in Spain. Further studies are needed to elucidate the life cycle, pathophysiology and phenotypic and genotypic characteristics of the organism in dogs in Nigeria.

Theileria equi

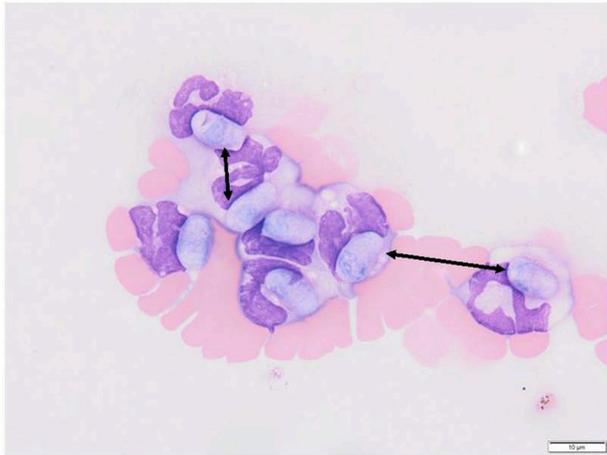
The equine piroplasms, *T. equi* and *B. caballi* have been detected in two symptomatic dogs in Croatia (Beck et al., 2009). The clinical significance of *T. equi* in dog is unknown. So far, human infections with any of the small *Babesia* spp. have not been reported.

Status in Nigeria

Microscopic detection of *T. equi* has not been reported in dogs in Nigeria. However, DNA of *T. equi* was amplified from the blood of dogs from Nigeria (Adamu et al., 2014). Although *T. equi* is morphologically similar to *B. gibsoni* (Purnell, 1981; Zahler et al., 2000) and since horses and dogs in Nigeria are known to live in close proximity with the likelihood for the exchange of vectors and hence pathogens as well, this finding this finding based on the detection of a short DNA fragment requires further investigation considering the established concept of host specificity of these parasites.



A



B

Fig. 4. A). Blood smear from a dog showing *Hepatozoon canis* gamont (arrow) in a leucocyte. Giemsa stain x100 objective. B). Blood smear from a dog showing *Hepatozoon canis* gamonts in leucocytes. Modified Wright's stain x100 objective.

Phylum: Apicomplexa
 Class: Conoidasida
 Order: Eucoccidiorida
 Suborder: Adeleorina
 Family: Hepatozoidae
 Genus: *Hepatozoon*

Hepatozoon canis (formerly *Leucocytozoon canis*). It was first described in the blood of dogs in India (James, 1905; Bentley, 1905). The gamonts of *H. canis* have an ellipsoidal shape and are found in the cytoplasm of neutrophils and monocytes (Baneth, 2011). *Hepatozoon canis* infection in dogs ranges from subclinical infection to severe and potentially lethal disease characterized by fever, anemia, emaciation and debility (Baneth and Weigler, 1997). This infection is frequently diagnosed by microscopic detection of intracellular gamonts in stained blood smears (Fig. 4A, B). The gamonts have an ellipsoidal shape and are found in the cytoplasm of neutrophils and monocytes (Baneth, 2011). There is no current report of human infection with *H. canis*.

Status in Nigeria

Hepatozoon canis gamonts have been detected in Giemsa stained blood smears from dogs in Nigeria using light microscopy (Aliu et al., 1976; Ezeokoli et al., 1983; Okubanjo et al., 2013). Furthermore, PCR targeting the 18S rRNA gene have amplified the DNA of *H. canis* from the blood of dogs in Nigeria (Sasaki et al., 2007; Kamani et al., 2013a).

Phylum: Chromadorea
 Class: Spirurida
 Order: Filarioidea
 Family: Onchocercidae
 Genus: *Acanthocheilonema* (*Dipetalonema*)

Acanthocheilonema dracunculoides (*Dipetalonema dracunculoides*).

Acanthocheilonema dracunculoides has been reported to infect domestic dogs primarily but has also been detected in the aardwolf (*Proteles cristatus*), the spotted hyena (*Crocuta crocuta*) and the red fox (*Vulpes vulpes*) (Sonin, 1985). The microfilariae of *A. dracunculoides* are unshathed, has no cephalic hook but posses a clear head space and a short attenuated tail free of nuclei that ends bluntly (Ortega-Mora et al., 1989; Marconcini et al., 1996). Infection occasionally presents with dermal clinical signs and lesions ranging from pruritus, alopecia, erythema to skin ulcers as well as other clinical signs such as ataxia, incoordination, cachexia, cyanosis, ascites and pleural effusion (Piercy, 1951; Bolio et al., 2002; Schwan and Schröter, 2006).

Status in Nigeria

Acanthocheilonema dracunculoides has been reported as a rare infection of dogs in Nigeria (Schillhorn van Veen, 1974).

4. Discussion

Tick-borne pathogens (TBPs) pose a great threat to the wellbeing of dogs and their owners globally (Chomel, 2011), especially where the capacity for timely and accurate diagnosis is lacking. In addition, globalization and global warming may result in the introduction of vectors or new TBPs to non-endemic areas (Harrus and Baneth, 2005; Colwell et al., 2011; Léger et al., 2013). Thus, the expanding spectra of TBPs and their vectors is a constant challenge to veterinarians who have to keep abreast with the changing disease patterns. Sensitive diagnostic tools are therefore indispensable for timely diagnosis, treatment and control of these diseases. The current practice where classical and molecular methods are concurrently used for the diagnosis and identification of TBPs of dogs is desirable as it combines the ease and cost effectiveness of light microscopy and other classical diagnostic methods and the sensitivity of molecular techniques (Solano-Gallego et al., 2016). When appropriately applied, they complement each other. Results generated by either method separately therefore should be interpreted or verified based on the available epidemiological information on a particular pathogen” Otherwise, there will be discordance between the reality of the disease and the published reports. This checklist is a synthesis of classical and molecular data on canine tick-borne pathogens in Nigeria intended to guide the medical and research community to make informed sound decision on their epidemiology. For example, *A. platys* was detected in blood smear of dogs for the first time in Nigeria by light microscopy in 2012 (Happi and Anita, 2012). A year later, this finding was corroborated by the amplification of the DNA of *A. platys* in the blood of dogs from Nigeria by PCR and sequencing (Kamani et al., 2013a). These results and the epidemiology of this pathogen suffice to state that the presence of this pathogen in Nigeria might have been either overlooked or misdiagnosed for years and the true prevalence therefore was probably underestimated. In the case of *E. ewingii* however, its detection in blood smears of dogs in Nigeria (Happi and Anita, 2012), has not been confirmed in any of the molecular studies conducted on blood samples of dogs from Nigeria (Sasaki et al., 2007; Kamani et al., 2013a, 2013b; Adamu et al., 2014; Happi et al., 2018). However, the detection of *E. ewingii*-DNA in dogs from Cameroon, a country that shares international border with Nigeria, suggests its possible occurrence in Nigeria (Ndip et al., 2005). Therefore, more studies are required to validate this finding and elucidate the potential presence and epidemiology of this pathogen in Nigeria.

Canine babesiosis is one of the most common tick-borne infections in Nigerian dogs. Diagnosis in most cases has been based on the morphological appearance of merozoites in blood smears according to the traditional classification of large and small piroplasms for *B. canis* and *B. gibsoni*, respectively. Accordingly, *B. gibsoni* was supposedly detected in blood smears of dogs in Nigeria (Oduye and Dipeolu, 1976; Idowu et al., 1977; Ezeokoli et al., 1983). However, several molecular studies on Nigerian dogs failed to detect *B. gibsoni* DNA in all screened samples (Sasaki et al., 2007; Kamani et al., 2010, 2013a; Adamu et al., 2014;

Happi et al., 2018). This is likely to be a misdiagnosis on the microscopic part since there are other small babesias that infect dogs in Nigeria and are difficult or impossible to morphologically differentiate from *B. gibsoni* (Birkenheuer et al., 2003; Miró et al., 2015). The detection of the DNA of *Theileria* spp. in the blood of dogs from Nigeria by PCR (Kamani et al., 2013a; Adamu et al., 2014) is similar to reports from other countries (Criado-Fornelio et al., 2003; Criado et al., 2006; Matjila et al., 2008; Yeagley et al., 2009; Iori et al., 2010), and deserves further studies in order to elucidate the epidemiology of this pathogen in Nigeria. For the large canine babesias in Nigeria, some confusion in nomenclature seems to dominate the literature (Oduye and Dipeolu, 1976; Idowu et al., 1977; Aliyu et al., 1990; Ahmed et al., 1994; Useh et al., 2003; Okoli et al., 2006; Omudu et al., 2007; Sasaki et al., 2007; Amuta et al., 2010; Kamani et al., 2010, 2013a; Adamu et al., 2012; Happi and Anita, 2012; Ifeoma, 2013; Okubanjo et al., 2013; Jegede et al., 2014; Opara et al., 2017; Happi et al., 2018). The current speciation of *B. canis* into three different species including *B. canis*, *B. rossii* and *B. vogeli* cannot be strictly determined by results obtained by light microscope studies since morphological differentiation between the three organisms is not possible. *Babesia canis* is vectored specifically by *Dermacentor* ticks in Europe (Mehlhorn et al., 1980; Uilenberg et al., 1989; Caccio et al., 2002; Duh et al., 2004), a tick genus that is absent in Nigeria. It follows therefore that the prevalence of *B. canis* in Nigeria should be null in the absence of the competent vector. However, there are a few reports of *B. canis* in Nigeria diagnosed by molecular methods (Kamani et al., 2010; Happi et al., 2018). The possible explanations for these findings being either importation of the parasite or the existence of another vector in Nigeria are yet to be identified. More investigation is required in this area. In Nigeria canine infection with *B. rossii*, the most pathogenic of the large babesias, appears to be more prevalent, 2–38% compared to *B. vogeli* 0.3–1% (Sasaki et al., 2007; Kamani et al., 2013a; Adamu et al., 2014). This is in agreement with earlier reports of canine babesiosis as a major cause of anemia in dogs from Nigeria (Abdullahi et al., 1990; Useh et al., 2003; Kamani et al., 2011). Several studies on the ectoparasites of dogs in Nigeria have reported *Rhipicephalus sanguineus* sensu lato (s.l.), the vector of *B. vogeli*, as the most predominant tick species infecting dogs and not *H. elliptica*, the vector of *B. rossii* (Dipeolu, 1975; Kamani et al., 2013a; Adamu et al., 2014). In fact, some studies did not find *Haemaphysalis* ticks on the examined dogs (Okoli et al., 2006; Adamu et al., 2012; Aquino et al., 2016; Opara et al., 2017). Whether this could be attributed to improper sampling methods or the rarity of *Haemaphysalis* spp. on dogs in Nigeria is a subject for further investigation. Importantly, the role of *Rhipicephalus* spp. in the transmission of *B. rossii* in Nigeria deserves some attention from the research community.

Canine hepatozoonosis caused by *H. canis* has been widely reported in Nigeria. Evidence for its cosmopolitan distribution in Nigeria has been demonstrated by microscopic examination as well as molecular analyses of blood samples of dogs from Nigeria. Reported prevalence ranges from 17 to 39% by microscopy and up to 41% by PCR (Aliu et al., 1976; Ezeokoli et al., 1983; Okubanjo et al., 2013; Kamani et al., 2013a; Opara et al., 2017), although several other studies failed to detect it (Oduye and Dipeolu, 1976; Okoli et al., 2006; Sasaki et al., 2007; Amuta et al., 2010; Adamu et al., 2012; Ifeoma, 2013; Adamu et al., 2014; Happi et al., 2018). Taking epidemiological factors into consideration, it appears that the distribution, true prevalence and economic impact of this parasite in Nigeria are yet to be fully ascertained.

Hemotropic *Mycoplasma* spp., are among the least studied TBPs of dogs in Nigeria due to their rarity and supposedly low pathogenicity. The earliest report of these pathogens in Nigeria was documented in the 1970s (Oduye and Dipeolu, 1976). Since then, there appears to be a lapse until recently when the DNA of *Mycoplasma* spp. was amplified and sequenced from the blood of Nigerian dogs. Three species; *M. haemocanis*, '*Candidatus Mycoplasma haematoparvum*' and '*Candidatus Mycoplasma haemobos*' have been detected in the blood of Nigerian

dogs, so far (Aquino et al., 2016; Happi et al., 2018). Risk factor analysis in one of the studies found a close association between ectoparasite prophylaxis and hemotropic mycoplasma spp. infection, but no association was found for age, gender, breed, health status, packed cell volume or management (Aquino et al., 2016). The clinical signs of canine hemoplasmosis in immunocompromised dogs mimic those of subacute babesiosis (Messick et al., 2002); therefore, clinicians should be aware of this and include it in the differential diagnoses of TBPs in Nigeria.

The tick-transmitted helminth *Acanthocheilonema dracunculoides* has been reported as a rare infection of dogs in Nigeria (Schillhorn van Veen 1974). Apart from the initial report there has not been any other documented clinical case in Nigeria. However, this should not be seen as the evidence of its absence considering the limitations of most of the sampling designs and diagnostic methods employed in survey studies in Nigeria. Generally, large-scale studies incorporating Remote Sensing and Geographic information system (GIS) technologies are required to elucidate the role of climate on the distribution and true prevalence of TBPs in dogs across the ecological zones of Nigeria.

In conclusion, a highlight of canine TBPs is presented in this article as a guide to the scientific and medical community in Nigeria. As a gradual transition from classical to molecular diagnostic and research methods is witnessed, there is a need to merge the "old classical" and the "new molecular" information in order to avoid confusion and allow detection of different infecting and coinfecting pathogens. The classical methods still remain the foundation and should not be jettisoned at the expense of the molecular methods. Additionally, caution should be exercised when reporting the findings from molecular studies bearing in mind that they deal with small fragments of the genome of an organism which can be subject to erroneous amplification and interpretation which is usually biased towards the dominant pathogen in the sample.

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Ethics statement

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