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ARTICLE

Current Status of Canine Babesiosis and the Situation in Nigeria: A Review

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SUMMARY

Canine babesiosis has been recognized globally as an emerging disease, thus the need to determine the exact species/subspecies and the clinical presentations of the disease associated with these species/subspecies. This review takes a concise appraisal of the current information on several aspects of the epidemiology, diagnosis, clinicopathology, treatment, prevention and control of the disease. Snapshot information on the status of canine babesiosis in Nigeria and the attention of veterinarians is drawn to the fact that there is more to canine babesiosis than just the disease caused by *Babesia canis.*

KEY WORDS: Canine babesiosis, Epidemiology, Clinicopathology, Control, Nigeria.

INTRODUCTION

Canine babesiosis, an important tick-borne infectious disease of dogs, has been described as an emerging veterinary problem worldwide (Duh *et al*., 2004; Irwin 2009). The disease is caused by an intraerythrocytic protozoan parasite of the genus *Babesia* with two main species; *Babesia canis* and *Babesia gibsoni* as the major culprits, even though *B. canis* has been shown to be more prevalent (Taboada and Merchant, 1991). The increasing number of canine *Babesia* species, geographical distribution, varying tick vectors and modes of transmission often result in motley of pathogenic and clinical presentations (Ayoob *et al.,* 2010); however, the general signs observed may range from a hyperacute shock-associated haemolytic crisis to an inapparent subclinical infection. Most dogs present with the acute form of the disease with clinical signs as fever, depression, anorexia, mucous membrane pallor, lymphadenopathy, splenomegally and general malaise (Birkenheuer *et al.,* 1999).

Advances in molecular biology has led to improvements in the diagnosis of the disease and also the nature of the parasites, the outcome of which is the discovery of new canine *Babesia* species and the inclusion of some *Theileria* species alongside the known *Babesia* species as being responsible for canine piroplasms.

TRANSMISSION, BIOLOGY AND MOLECULAR SPECIATION

Transmission of *Babesia* parasite to the canine host is by the bite of specific ixodid tick vectors of the genus *Rhipicephalus* (*Rhipicephalus sanguineus*), *Haemaphysalis* (*Haemaphysalis leachi*, *H. bispinosa* and *H. longicornis*) and *Dermacentor* (*Dermacentor reticulatus*). Thus the specie of *Babesia* prevalent in a particular area is influenced by the presence of the specific tick vector in that geographical area (Birkenheuer *et al.,* 1999; Matjila *et al*., 2004).

Birkenheuer *et al.* (1999), Miyama *et al*. (2005) and Jefferies *et al.* (2007a) have suggested a direct dog to dog transmission of *B. gibsoni.* They suspect that the parasite is transmitted through blood and saliva when an infected dog with oral abrasions bites a naïve dog during fights. This bite-blood-saliva transmitted *B. gibsoni* infection has been associated with breeds of dogs renowned for aggression, such as the American Staffordshire/Pit Bull terriers in the USA and the Tosa breed in Japan. Transplacental transmission has been reported in puppies as young as 3 days old while experimental infection has led to stillbirths or death of puppies 6 weeks post partum (Meinkoth *et al.,* 2002; Fukumoto *et al.,* 2005; Jefferies *et al.,* 2007a).

Babesia parasites have traditionally been

differentiated phenotypically based on their appearance in stained blood smears particularly using the size of their piroplasms as either small (*B. gibsoni:* 1μm ×3.2μm) or large (*B. canis*: 3μm ×5μm) (Passos *et al*., 2005).

Babesia canis trophozoites are large bilobed piriform organisms of 4-5μm in length and 2-4μm in diameter and appear as round, oval or ringed-shaped inside erythrocytes (Lewis *et al*., 1996). Three subspecies of *Babesia canis* have been recognized namely; *Babesia canis canis, Babesia canis rossi* and *Babesia canis vogeli*, all of which are identical morphologically but differ in geographical distribution, antigenic properties, virulence and tick vector (Uilenberg *et al*., 1989; Caccio *et al*., 2002). However, two new large *Babesia* species which have not been given a taxonomic status have been reported in North America by Birkenheuer *et al*. (2004), and another in Brazil that seems to be different from *Babesia* and *Theileria* because of its intraendothelial stage (Loretti and Barros, 2005). Also Baneth *et al*. (2004) are proposing new *Babesia canis* subspecie (*Babesia canis presentii*) which infects cats. This proposed subspecie shows a high molecular similarity of 18S RNA genes with *B. canis* but is quite smaller in size. The distinct characteristics of each of these subspecies in terms of their geographical distribution, virulence, vector, pathogenicity, and antigenic properties have informed the decision of several authors to advocate for separate taxa for them (Schetters *et al.*, 1997; Zahler *et al*., 1998; Baneth *et al*., 2004).

Babesia gibsoni which measures about 1-3.2μm and appears singly in erythrocytes initially was the only small piroplasm of dog identified. However, recent advances in molecular techniques have revealed that at least three morphologically similar but genetically distinct small *Babesia* parasites of dogs exist, and these include; *Babesia gibsoni* measuring 1-3.2μm and appears as either piriform or ring form, *Babesia conradae* which measures 1-2.5μm, and appears as ring, piriform, tetrad, amoeboid or anaplasmoid forms at the intraerythrocytic merozoite stage, with an apical complex and rhoptries under transmission electron microscope and *Theileria* (*Babesia microti-like*) *annae* which has a tetrad form at the merozoite stage, an apical complex and an exoerythrocytic stage (Thomford *et al.*, 1993; Kjemtrup *et al*., 2000; Goethert *et al.,* 2003; Kjemtrup *et al*., 2006; Matjila *et al.,* 2007). Note that under light microscopy the intraerythrocytic stage of *Babesia* spp. is indistinguishable from *Theileria* spp. and can only be seperated on the basis of certain life-cycle stages and transovarial passage within the tick vector (Uilenberg, 2006). Other theileria species that have been recorded in dogs using 18S rRNA gene includes *Theileria equi* and *Theileria annulata* (Criado-Fornelio *et al*., 2003b). In essence, the previous notion of Babesia spp being solely responsible for canine piroplasmosis is no longer tenable especially with the advent of molecular techniques, sequencing and phylogenetic analysis. Current publications on the use of these methods in the analysis of the small sub-unit ribosomal RNA (ssrRNA) of canine piroplasms shows that they belong to three clades ; 'true' Babesia sp. (*B. canis* and *B. gibsoni*), *B. microti* clade (*Theileria annae*) and the Theileria-like group (*B. conradae*). These observations have introduced a major change in the approach to the epidemiology of babesiosis in dogs. Also, the recent identification of unusual piroplasms *(Theileria (Babesia) equi, Theileria annae, Theileria annulata, Babesia caballi)* other than *Babesia canis* sp and *Babesia gibsoni* in dogs is worrisome and may be responsible for the non-responsive treatment of canine babesiosis cases in the clinics using the standard dosage of imidocarb dipropionate at 3 mg/kg (Criado-Fornelio *et al.,* 2003, Criado *et al*., 2006, Fritz, 2010).

Table I: Summary of Piroplasms identified in dogs from different areas of the world

Species	Size	Areas reported	Tick vector	References
Large Babesia spp				
B. canis canis	3 µm- 5 µm	Europe and Asia	*Dermacentor reticulatus* *Rhipicephalus sanguienus*	Øines *et al*., 2010 Adaszek and Winiarczyk, 2008
		Nigeria	,, ,,	Kamani *et al*., 2010
B. canis rossi	3 µm-5 µm	South Africa Nigeria	*Haemaphysalis elliptica* unknown	Matjila *et al*., 2009 Kamani *et al*., 2010
B. canis vogeli	3 µm -5 µm	Africa, Asia,Australia,	*Rhipicephalus sanguineus*	M'ghirbi and Bouattour, 2008
		Europe, South America and USA.		Beck *et al.,* 2009
New Large Babesia spp. (Coco)	2 µm-5 µm	North Carolina	Unknown	Birkenheuer *et al.*, 2004 Lehtinen *et al*., 2008
Babesia caballi	2µm–4 µm	France, Croatia	Unknown	Beck *et al.,* 2009 Fritz 2010
Small Babesia spp				
Babesia gibsoni	1 µm-3.2 µm	Africa, Asia,Australia, Europe, South America and USA.	*Haemophysalis leachi, Rhipicephalus sanguineus*	Varshney *et al* ., 2008 Hartelt *et al*., 2007
Babesia conradae	1 µm- 2.5 µm	California (USA)	Unknown	Kjemtrup *et al.,* 2006
Theileria *(Babesia microti)* annae 2000	1 µm-2.5 µm	Northwestern Spain	*Ixodes hexagonus?*	Zahler *et al.,* 2000 Camacho *et al*., 2003 Garcia 2006
Theileria (Babesia) equi	1 µm-2.5 µm	Spain	Unknown	Mehlhorn and Schein, 1998 Criab - Fornelio *et al.,* 2003 a,b
Unnamed Theileria sp		South Afric	Unknown	Matjila *et al*., 2008c
Theileria annulata	1 µm-2.5 µm	Spain	Unknown	Criado *et al*., 2006

EPIDEMIOLOGY

Tick vectors and distribution

The distribution of canine *Babesia* parasites is world wide and is dependent on the presence of the specific tick vector responsible for their transmission, such that the *Babesia canis vogeli* which is transmitted by *Rhipicephalus sanguineus* is seen in North Africa, South America, Southern and Eastern Africa, and European countries (Duh *et al*., 2004; Matjila *et al*., 2004; Oyamada *et al*., 2005; Eiras *et al*., 2008; M'ghirbi and Bouattour, 2008); *Babesia canis rossi* transmitted by *Haemaphysalis elliptica* (Apanaskevich *et al*., 2007) is seen in South Africa and Sudan (Oyamada *et al*., 2005; Matjila *et al*., 2008a; Matjila *et al.,* 2008b), whereas *Babesia canis canis* transmitted by *Dermacentor reticulatus* is distributed mainly in Europe (Caccio *et al*., 2002; Duh *et al*., 2004; Solano-Gallego *et al*., 2008). *Dermacentor reticulatus* is an exophilic and ditropic tick found mainly in forests, but adapted to suburban habitats and is the major vector species of canine babesiosis in France (Bourdoiseau, 2006).

The small Babesia (*Babesia gibsoni, Babesia conradae* and *Babesia (Theileria) annae)* which has three genetically distinct entities are found mainly in Asia (Farwell *et al.,* 1982; Fukumoto *et al.,* 2001; Song *et al.,* 2004; Miyama *et al.,* 2005; Matjila *et al.,* 2007), North America (Conrad *et al.,* 1991; Kjemtrup *et al.,* 2006a; Kjemtrup *et al*., 2006b) and Europe (Kjemtrup, 2000; Camacho-Garcia, 2006). *Babesia conradae* has been shown to be closely related to piroplasm isolates from wildlife and humans and distributed around the California area of USA while *Babesia (Theileria) annae* is similar to *Babesia microti* and *Theileria equi* and occurs mainly in Spain (Kjemtrup *et al.,* 2006; Camacho-Garcia, 2006). The vector for this emergent canine infection has not been described, although *Ixodes hexagonus* is suspected based on their presence upon dogs in North-West of Spain and the relative absence of other ticks (Dixit *et al.,* 2010). What the above information means is that the detection of a small piroplasm in the erythrocytes of dogs is not indicative of *Babesia gibsoni,* for the simple reason that the small piroplasms share similar morphology. Thus, *B. gibsoni* cannot unequivocally be differentiated from *B. equi* or *B. microti* based on size, shape or location in the erythrocytes (Conrad *et al.,* 1992; Zahler *et al.,* 2000).

Ticks responsible for the transmission of small piroplasms in dogs are mainly *Rhipicephalus sanguineus*, an endophilic and monotropic tick adapted to premises, habitations, kennels and all biotopes in which man and dogs cohabit (Higuchi *et al*., 1995; Inokuma *et al*., 1998; Bourddoiseau, 2006), *Haemaphysalis bispinosa* (Grooves and Yap, 1968) and *Haemaphysalis longicornis* (Higuchi *et al*., 1991)(Table I). Canine piroplasmosis is a disease of young dogs even as young as 3weeks (Harvey *et al*., 1988), however older dogs coming from a Babesia-free zone can develop the disease on contact with an infected tick during a brief visit in an endemic zone, with the highest peak of the disease coinciding with the period most favourable for the tick vector activity which is usually at the beginning of the rains in tropical Africa and autumn/spring in temperate Europe (Bourdoiseau, 2006).

Breed Susceptibility

Although breed susceptibility and specificity has not been established for canine babesia infection, several authors have associated certain *Babesia* species with some dog breeds as espoused by Breitschwerdt *et al.* (1983) ; Yamane *et al.* (1994) and Birkenheuer *et al.* (2005) who reported a 50-55% seropositivity of *B. canis vogeli* infection in Greyhounds breeds whereas susceptibility rate for *B. gibsoni*-seropositive dogs among the American Staffordshire and Pit Bull Terriers breeds was put at 15-93% (Macintire *et al.,* 2002; Birkenheuer *et al.,* 2003b; Birkenheuer *et al.,* 2005).

DIAGNOSIS

Babesia infections are traditionally diagnosed based on the detection of the parasites in thin blood smears stained with Giemsa, Romanowsky and field stains under a microscope. The blood smears prepared from capillary blood and buffy coat readily reveals the parasites since the parasitized erythrocytes tend to sludge in the capillaries and also preferentially parasitize the reticulocytes over the mature red blood cell (Mattia *et al.,* 1993; Bohm *et al.,* 2005).

Identification of the parasites relies on the morphology of the intraerythrocytic forms using their size; however, this method is affected by its limited sensitivity and the subjectivity of the observer especially during asymptomatic and chronic infections when the parasitaemia is low and usually undetected by microscopy (Song *et. al*., 2004; Miyama *et al*., 2005).

Fukata *et al*. (1996) and Yamasaki *et al*. (2008) have developed a flow cytometry to diagnose and evaluate the level of *B. gibsoni* parasitaemia *in vivo* and *in vitro* using a fluorescent nucleic acid stain SYTO16 which has been shown to be rapid and reliable. Other serological tests that have been used for the diagnosis of canine babesiosis are indirect fluorescent antibody technique (IFAT) and enzyme linked immunosorbent assay (ELISA) technique (Yamane *et al*., 1993; Fukumoto *et al*., 2004); however, these serological tests are known to show cross-reaction between different species of *Babesia* and do not differentiate acute from chronic infection thus making them non-specific (Yamane *et al*., 1994; Irwin, 2007). Cross-antigenicity seen in the *B. canis* subspecies is thought to be responsible for vaccine failures in the field as observed by Uilenberg *et al.* (1989) and Schetters *et al.* (1995), thus the possibility of developing potent vaccines against canine babesiosis will be dependent on the proper differentiation of the different subspecies.

Polymerase chain reaction (PCR) with its several variations as a diagnostic tool for *Babesia* parasites has been evaluated and found suitable because of its sensitivity and specificity which is estimated to approach 100% (Jefferies *et al.,* 2007b), and its ability to detect past, asymptomatic and current infections (Birkenheuer *et al*., 2003a; Song *et al*., 2004; Miyama *et al*., 2005). This method lays emphasis on the amplification of the babesia DNA instead of the anti-babesial antibodies, thus making it a very reliable diagnostic tool in acute, peracute, and chronic infections most especially in immunocompromised and young dogs (Fukumoto *et al.,* 2001). It has also been found quite useful in epidemiological studies for the identification of new subspecies and for the differentiation of close and genetically distant *Babesia* species (Ano *et al.,* 2001; Birkenheuer *et al*., 2003a).

Recent advances in molecular techniques have seen an avalanche of methods used to diagnose these parasites. Some of these methods which usually incorporate PCR in their procedures

include; Reverse line Blot hybridization (Matjila *et al*., 2004; Matjila *et al*., 2008) and Restriction fragment length polymorphism (Conrad *et al.,* 1992; Carret *et al*., 1999; Solano-Gallego *et al.,* 2008) amongst others. To achieve optimal diagnostic accuracy, Irwin (2009) advocates a combination of IFAT and PCR even though he was silent on the cost effectiveness of such test especially in developing countries. For subspecies differentiation and phylogenetic analysis, sequencing of the full-length small subunit ribosomal RNA (ssrRNA) is the method of choice since there in no ambiguity in the results generated from using it (Caccio *et al*., 2002; Eiras *et al*., 2008).

CLINICOPATHOLOGICAL FINDINGS

The clinical and pathological presentation of canine babesiosis varies and is dependent on the species/subspecies responsible for the infection; however, the classical presentations often include: Thrombocytopenia, febrile syndrome (Fever, anorexia, depression, dehydration) and haemolytic syndrome (anaemia, bilirubinuria, haemolysis) in acute cases while the chronic form corresponds to prolonged convalescence characterized by depression (Bourdoiseau, 2006; Solano-Gallego *et al*., 2008). In terms of severity of infections associated with the subspecies of *Babesia canis*, evidence shows that *Babesia canis rossi* is the most virulent with haemolytic and inflammatory responses (Reyers *et al*., 1998), *Babesia canis canis* shows a transient parasitaemia (<1%) associated with congestion of internal organs (Schetters *et al*., 1997), where as *Babesia canis vogeli* lead to a relatively mild infection, often without clinical signs or where present may not be homogenous (Caccio *et al*., 2002; Solano-Gallego *et al*., 2008). Infections due to *Babesia gibsoni* are usually associated with splenomegally, hepatomegally, haemolytic anaemia and severe thrombocytopenia (Conrad *et al*., 1991; Matjila *et al*., 2007). Clinical signs of *Babesia conradae* are similar to those of *B. gibsoni*, but *B. conradae* infection is more pathogenic with pronounced anaemia, higher parasitaemia and lymphadenopathy (Kjemtrup and Conrad, 2006). *Babesia* (*Theileria*) *annae* infection is characterized by severe regenerative anaemia and thrombocytopenia, azotemia is seen in many cases, while the presence of hyaline and granular casts in the urine of infected dogs is suggestive of renal involvement in the disease (Camacho-Garcia, 2006).

TREATMENT

Drugs that have been used for the treatment of canine babesiosis (Atavaquone, azithromycin, diminazene aceturate, phenamidine isethionate, pentamidine, parvaquone, niridazone and trypan blue) are known to be unable to completely eliminate the parasites and the disease, but can only reduce the severity of the clinical signs and the mortality Birkenheuer *et al*. (1999). These drugs show varying degrees of success rates either alone or in combination in terms of eliminating the parasites or reducing the parasite load as was adduced by Birkenheuer *et al*. (1999) and Matjila *et al.,* 2007. Although no known drug(s) has the capacity to treat infection due to *Babesia canis*, the report of Birkenheuer *et al.* (2004b) showed that a combination of azithromycin and atovaquone therapy is able to treat *Babesia gibsoni* infections in dogs successfully without infected erythrocytes being seen in capillary blood smear; also blood from dogs with this combination therapy was shown to be negative on PCR assay for about 4 months. This information seem more encouraging when compared to single therapy with atovaquone in which *B. gibsoni* parasite DNA were intermittently detected in the blood of experimentally infected dogs 33 days after the last treatment (Matsu *et al.,* 2004), thus supporting the assertion by Choidioni *et al.* (1995) and Wittner *et al.* (1996) that recurrence of disease and decreased sensitivity to protozoa parasites (*Plasmodium falciparum* and *Babesia microti*) occurred following therapy with atovaquone alone.

Although research has shown that treatment of canine babesiosis due to *B. gibsoni* with diminazene and or imidocarb is ineffective (Birkenheuer *et al*., 1999; Stageman *et al.,* 2003), it is imperative to state that Imidocarb has the capacity to stop the multiplication of the intraerythocytic parasites and also allow the persistence of several parasites in order to induce immunity and as such are desirable for the treatment of infection due to *B. canis* (Brandao *et al*., 2003; Bourdoiseau, 2006).

Most if not all the babesiacidal drugs are toxic to the host and are used with the utmost caution.

Toxicity with these drugs is expressed in form of CNS disorders (diminazene); vomiting, colic and diarrhoea alongside hepatic, renal or vascular complications (imidocarb). Irrespective of the drug(s) used for the treatment of canine babesiosis, it is recommended that supportive therapy using intravenous fluids, corticosteroids and blood transfusion be used alongside.

PROPHYLAXIS

This involves chemoprophylaxis in which acaricides (amitraz, fipronil, permethrin) are applied topically to reduce the tick burden on the animal before a high dose of drug (Imidocarb) is injected intramuscularly. This allows the residual effect of the drug to offer protection for about 4-6 weeks and is especially useful in animals with a history that contraindicates vaccination (Uilenberg *et al*., 1981; Bourdoiseau, 2006); and vaccination which involves the subcutaneous injection of soluble parasite antigens (SPA) vaccines prepared from *in vitro* cultures of *B. canis canis* and *B. canis rossi*. Currently, vaccines (Nobivac® and Pirodog®) containing soluble parasite antigens (SPA) from cultures of *Babesia canis* and *Babesia rossi* have been developed and are commercially available in Europe to protect dogs against heterologous *Babesia canis* infection (Bourdoiseau, 2006; Schetters *et al.,* 2006).

Schetters *et al.* (2006) proved that this vaccine induces protective immunity against clinical babesiosis resulting from heterologous challenge from 3 weeks after booster vaccination onwards, and remains effective for about 6 months. The administration of SPA vaccines (Nobivac® and Pirodog®) requires that the animal be in good health, at least 5 months old, not given to pregnant females and not administered concurrently with other vaccines apart from rabies and leptospirosis vaccines (Bourdoiseau, 2006).

THE NIGERIAN PERSPECTIVE

Canine babesiosis is common in Nigerian dogs due to prevalence of the tick vector, *Rhipencephalus sanguineus* (Abdullahi *et al*., 1990). Diagnosis is usually by microscopy which is based on morphological classification of the parasite either as large (*Babesia canis*) or small (*Babesia* gibsoni), relating to their size within the erythrocytes (Adeyanju and Aliu, 1982; Bobade *et al.,* 1989). Serological diagnosis by Enzyme Link Immunosorbent assay (ELISA) is rarely conducted on clinical cases, while molecular studies are occasional (Sasaki *et al*., 2007; Kamani *et al*., 2010). The few molecular studies that were undertaken provided information on subspecies of *Babesia canis* present in Nigerian dogs. Sasaki *et al*., (2007) reported the presence of *Babesia canis rossi* and *B canis vogelli* in 2.0% and 0.3% Nigerian dogs respectively using nested PCR and sequence analysis. Kamani *et al*. (2010) using specific PCR for Babesia spp. and DNA sequencing reported for the first time in an untraveled Nigerian dog the presence of *Babesia canis canis* on the African continent as a co-infection with *Babesia canis rossi,* also a rare occurrence. Prevalence of the disease in Nigeria has ranged between 2% and 43.6% and has been reported in all the states of the federation (Dipeolu, 1975; Bodade *et al.*, 1989; Amuta *et al*., 2010).

DISCUSSION

Canine babesiosis, an important tick-borne infectious disease of dogs is regarded as an emerging veterinary problem worldwide. The disease in Nigeria has been recorded in several publications, however recent reports using molecular techniques shows the presence of all the three large Babesia subspecies (*Babesia canis, Babesia vogeli and Babesia rossi*) in the country (Sasaki *et al*., 2007; Kamani *et al*., 2010), an information that is of grave consequence to the animals and of importance to the veterinary practitioners considering the various clinical presentations of the species. Infact the clinical report of *Babesia rossi*, (the most virulent of the species) and *Babesia canis* is of note since their presence, endemicity, and proven tick vectors (*Haemaphysallis elliptica* and *Dermacentor* spp) are associated with Eastern and Southern Africa, and Europe (Uilenberg, 1989; Oyamada, 2005; Matjila, 2008b).

The reports of these Babesia species in the country may not be surprising in view of the unregulated importation of exotic breeds of dogs from South Africa, and other areas of the world that are endemic with several species of the large *Babesia,* Babesia *gibsoni*-like parasites and *Theileria* sp. This is because translocation of infected dogs from Babesia endemic areas to free areas has been implicated as a major factor in the

spread of the parasite (Conrad *et al.*, 1991).

Nigeria does not consider canine babesiosis as a control disease and as such does not enforce the pre-import blood testing or the post-importation blood testing involving serological and molecular testing from dogs entering the country. The danger of the above laxity in enforcing surveillance and import control is the possible introduction of foriegn tick vectors and their associated parasites/diseases into the country.

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