

# A review of some tick-borne pathogens of dogs

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**ABSTRACT:** Ticks and tick-borne diseases have great economic and medical importance worldwide and they affect both animal and human health by sucking blood and also transmitting protozoan, bacterial, rickettsial, spirochetal and viral agents. Ticks can live in all regions of the world except Polar Regions. Tick-borne encephalitis Virus, Babesiosis, Anaplasmosis, Ehrlichiosis, and Hepatozoonosis are important tick-borne diseases of dogs and they influence a great variety of other domestic and wild animals. Ticks are ectoparasites of mammals, birds and reptiles specializing as obligate blood sucking permanent ectoparasitic arthropods. As vector of important haemoparasitic disease, tick surpasses all other arthropods, apart from mosquitoes as diseases agents of humans and lead to poor performance of the animals. One or more of the approximately 840 known species of ticks are found in most terrestrial regions of the earth. Two major families of ticks are ixodidae (hard bodies ticks) and argasidae (soft bodies ticks), with ixodidae being the largest and most important family. These vectors such as *Rhipicephalus sanguineus* and *Ixodes ricinus* which are endemic in Nigeria, have affected the performance of dogs which are major human companions through their activities such as blood sucking, disease transmission, biting effect and causing listlessness through itching and scratching. The important pathogens dealt with in this study, include tick-borne encephalitis virus, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Hepatozoon canis* and *Babesia canis*. This review aims at to create better awareness on ticks and tick-borne pathogens of dogs providing latest knowledge on prevention and control of this menace.

**Key words:** Dogs and tick vectors, Encephalitis virus, epidemiology and harmful effects, Haemoparasites, molecular detection, preventive measures, Rickettsiae,

## INTRODUCTION

Ticks are the second most common blood-feeding parasites after mosquitoes. They do not only destroy blood cells thereby causing anaemia but also transmit and carry different kinds of protozoa, viruses and bacteria some of which may cause tick-borne diseases (TBDs). (de la Fuente et al., 2008; Otranto et al., 2014). These tick-borne diseases not only include various existing infectious diseases, but also comprise of both emerging and reemerging infectious diseases. An instance of such emerging infectious disease is fever characterized by thrombocytopaenia, this was reported in China and was

seen to be endemic and it poses a serious threat to both animal and human health (Parola et al., 2005; Yu et al., 2011). However, in the last few decades, the number of reported cases of infection with new TBDs in humans and animals has increased (Kernif et al., 2016).

Transmission of tick-borne pathogens occurs from tick to their hosts but both trans-ovarially (i.e. the eggs acquire infection from the egg-laying female) and trans-stadially (i.e. both larvae and nymphs are able to transmit the pathogen to the next developmental stage) (Beugnet, 2002). Attaching of ticks firmly to their hosts, facilitates the

effective blood feeding, the transmission of pathogens but also the spread of both ticks and microorganisms to different geographical habitats via migrating animals or travelling pets (Kenny et al., 2004b).

Owing to the fact that some TBDs in humans are zoonoses and are of grave economic and medical importance, it is necessary to expatiate more on the tick-borne pathogens in pets particularly of dogs.

The most common tick-borne protozoan pathogens of dogs are *Babesia* and *Hepatozoon* (Homer et al., 2000; Baneth et al., 2003). These haemoparasites live in mammalian blood cells and cause severe diseases and sometimes death in infected animals (Schnittger et al., 2012). Specifically, *Babesia gibsoni*, *Babesia canis*, *Babesia rossi*, and *Babesia vogeli* are causative agents of canine babesiosis (biliary fever). *B. gibsoni* is distributed in Asia, North America, Europe, and northern and eastern Africa (Farwell et al., 1982; Jefferies et al., 2003; Lobetti, 1998). *B. canis* is transmitted by *Dermacentor reticulatus*, which is prevalent in Europe (Solano-Gallego and Baneth, 2011). The other two species, *B. rossi* and *B. vogeli*, are mainly transmitted by *Haemaphysalis leachi* and *Rhipicephalus sanguineus* sensu lato (s.l.), respectively (Apanaskevich et al., 2007; Criado-Fornelio et al., 2003). The distribution of *B. rossi* is restricted to sub-Saharan Africa, while that of *B. vogeli* is worldwide (Europe, Africa, Asia, South and North America) (Oyamada et al., 2005; Matjila et al., 2008). *Hepatozoon canis* and *Hepatozoon americanum* are the agents of canine hepatozoonoses that range from being asymptomatic with low levels of parasitaemia to a severe life-threatening illness characterised by high levels of parasitaemia, fever, anaemia, and lethargy (Baneth et al., 2000; Baneth et al., 2003). These two *Hepatozoon* species are genetically and geographically distinct (Baneth et al., 2000). *Hepatozoon canis* is an apicomplexan protozoan. Its transmission differs from other tick-borne pathogens because infection of the dog takes place by ingestion of a tick containing the parasite. Its vector species is *R. sanguineus*. *H. canis* is distributed in Africa, southern Europe, the Middle East, and Asia (Baneth and Vincent-Johnson, 2006) and is mainly transmitted by *R. sanguineus* s.l. and *Haemaphysalis longicornis* (Dantas-Torres et al., 2012; Murata et al., 1995). *H. americanum* is found in the Americas and is transmitted by *Amblyomma maculatum* (Mathew et al., 1998). Recently, new *Hepatozoon* spp. were reported in dogs and wildlife in Turkey and Brazil (Aydin et al., 2015; Soares et al., 2017).

*Anaplasma* and *Ehrlichia* species are obligate intracellular bacteria that belong to the family of Anaplasmataceae. These tick-borne pathogens infect humans and animals all over the world. *Anaplasma platys* is primarily found in dogs with cyclic thrombocytopaenia (Harvey et al., 1978). In addition, new *Anaplasma* species that are closely related to *Anaplasma phagocytophilum*, which causes human granulocytic anaplasmosis, have been detected in canine blood (Inokuma et al., 2005).

*Ehrlichia canis* is the causative agent of canine ehrlichiosis, which is transmitted by *R. sanguineus* s.l. (Groves et al., 1975; Aguiar et al., 2007). While *E. canis* was initially thought to be pathogenic in canines only, it was eventually also detected in human patients with the typical clinical findings of ehrlichiosis (Perez et al., 2006).

Tick-borne encephalitis virus (TBEV) (Flaviviridae) is primary pathogenic to humans but also infects dogs (Shaw et al., 2001). Seroprevalence of the virus has been found to be higher in dogs because they come into contact with the infected vector ticks (mainly *Ixodes ricinus*) more frequently than humans. However, the risk for developing clinical tick-borne encephalitis (TBE) in tick-infested dogs in an endemic area seems to be rather small for unknown reasons. Altogether less than twenty clinical TBE infections of dogs have been reported in Austria, Germany, Sweden and Switzerland (Beugnet, 2002).

Therefore, the aim of this review is to help create a better awareness on ticks and tick-borne pathogens of dogs by providing latest knowledge of the pathogens, prevention and control of this menace.

## CANINE EHRLICHIOSIS

Ehrlichiosis is also known as canine rickettsiosis, canine hemorrhagic fever, canine typhus, tracker dog disease, and tropical canine pancytopenia (spelling) is a tick-borne disease of dogs usually caused by the organism, *Ehrlichia canis*. Humans can become infected by *E. canis* and other species after tick exposure.

## Etiology

*Ehrlichia* is a *Rickettsia* of the Anaplasmataceae family, Gram-negative intracytoplasmic bacteria that invade and multiply within leukocytes and platelets in the peripheral blood of various species of domestic and wild mammals. It causes Ehrlichiosis (Borjesson, 2000), a world-wide distributed zoonosis (Perez et al., 2005), concentrated in tropical and subtropical regions due to the geographical distribution of its vector tick Ixodidae, *R. sanguineus* which can rarely infect humans (Dantas-Torres et al., 2006). In Brazil, this seems to be the main vector for *E. canis* in urban areas, although in rural areas human infection seems to be related to the genus *Amblyomma* (Labruna and Pereira, 2001).

Several species of *Ehrlichia* infect dogs such as *Anaplasma platys*, *E. equi*, *E. ewingii*, *E. risticii*, *E. chaffeensis*, *E. sennetsu* and *E. canis*. The latter being the main species that infects dogs producing several clinical symptoms (Stiles, 2000) - fever, anorexia, vomiting, loss of weight, enlargement of the liver, spleen and lymph nodes, epistaxis, hemorrhage and thrombocytopenia (Moreira et al., 2003).

## Transmission Cycle

The brown dog tick (*R. sanguineus*) acts as the primary vector of *E. canis* transferring the pathogen between hosts during blood meal. Dogs both domestic and wild serve as reservoir hosts for this pathogen and are the primary hosts of brown dog ticks. Brown dog ticks become carriers of the pathogen when they take a blood meal from a rickessemic dog. Stored in midgut and salivary gland of an infected tick, *E. canis* is transferred via the saliva of ticks carrying the pathogen to host during blood meal (Bowman et al., 2009). If infected while in the larval stage, the ticks retains the pathogen through the next two life instars and can inoculate hosts during blood meals in both the nymph and adult stage in transstadial transmission. Because of the vector of *E. canis* uses canine species as a primary hosts, this organism is most commonly associated with dogs, but human cases have been reported.

## Epidemiology

Serologic evidence of previous studies around the world indicated that *E. canis* is present among dogs throughout all continents with a prevalence ranging from 30 to 80% in some countries of Africa (Azzag et al., 2015; Inokuma et al., 2006; Kelly et al., 2004; Ndip et al., 2005; Davoust et al., 2006), while in some Asian countries it was 0.2 to 30% (Inokuma et al., 1999; Stich et al., 2008). In Europe, a prevalence ranging from 2 to 50% have been found. A study realized in the USA detected most often *Ehrlichia* antibodies in dogs in the Southeast, with 1.3% of samples testing positive, whereas other regions showed lower numbers ranging from 0.3 to 0.6%. (Bowman et al., 2009). Depending on technique used, different results were found in Oklahoma, where the prevalence of *E. canis* was 10.8% by serology, and 3.1% by the polymerase chain reaction (PCR) (Murphy et al., 1998). Among dogs belonging to the U.S.A. military forces, seropositivity to *E. canis* ranged from 8% in cold zones (above 45° latitude) to 24% in temperate places (between 40 and 45° latitude); a 13% prevalence was found in tropical zones (below 40° latitude) (Keefe et al., 1982). Several studies on *E. canis* prevalence have been realized in Brazil. In Grenade, 43.8% of dogs tested were positive for *E. canis* (Yabsley et al., 2008) while in Mexico, studies performed in the southern area found 44% of seropositive dogs to *E. canis* with ELISA testing (Rodriguez-Vivaz et al., 2005). Although much have been said about the presence of this disease in Mexico, currently there are no reports of it in the northeast region; therefore, the goal of the present study was to estimate the seroprevalence of ehrlichiosis in dogs from the city of Monterrey, located in this part of Mexico.

## Clinical signs

There are three stages of ehrlichiosis:

### Acute stage

Symptoms present around 1-3 weeks after bite from infected tick. Enlarged lymph nodes, weakness, lethargy, depression, lack of appetite, difficult respiration and limb edema.

### Sub-clinical stage

The organism may be present from months or years without clinical symptoms.

### Chronic stage

Abnormal bleeding, nose bleeding, severe weight loss, fever, difficulty in breathing due to inflammation of the lungs, joint inflammation and pain, seizures in some animals, lack of coordination, head tilt, anemia, kidney failure and paralysis.

## Pathology

Studies have shown that partial feeding of nymphs infected as (tick) larvae with *E. canis* is one possibility for these ticks to be able to infect dogs. The *E. canis* organisms are found in the midgut and salivary glands of infected adult ticks. The tick can transfer the organism from its saliva to the dog while feeding. Once inside the dog, they infect monocytes capable of spreading throughout the lymphatic system, including the spleen and liver. This will lead to an abnormal increase in cell size and number, known as hyperplasia. Replication and cell division can lead to further spreading of the bacterium throughout the host and eventually leads to anaemia and an increase in platelet count. Once the dog is infected, several signs of ehrlichiosis include marked thrombocytopaenia, pyrexia, reduction in the packed cell volume and the presence of *E. canis* in peripheral blood mononuclear cells. If the infection is not treated, the disease can take on a chronic form, where bone marrow will fail to develop along with anaemia, making the dog more susceptible to other infections. The dog will respond poorly to treatment at this stage and soon die of hemorrhage (Watanabe et al., 2004).

## Diagnosis

Diagnosis is achieved most commonly by serologic testing of the blood for the presence of antibodies against the *Ehrlichia* organism. Many veterinarians routinely test for the disease, especially in enzootic area. During the acute phase of infection, the test can be falsely negative because the body will not have had time to make antibodies to the infection. As such, the test should be repeated. PCR test can be performed during this stage to detect genetic

material of the bacteria. The PCR test is more likely to yield a negative result during the subclinical and chronic disease phases. In addition, blood test may show abnormalities in the numbers of red blood cells, white blood cells, and most commonly platelets, if the disease is present. Uncommonly, a diagnosis can be made by microscopical examination of blood smear for the presence of the *Ehrlichia morulae*, which sometimes can be seen as intracytoplasmic inclusion bodies within a white blood cell (Dumler et al., 2007).

## Treatment

*E. canis* is commonly treated via chemoprophylaxis with antibiotic, doxycycline. If diagnosed before the clinical stage, *E. canis* is almost completely curable but prolonged presence of the pathogen leads to hemorrhage which usually results in the death of the patient (Bowman et al., 2009).

## ANAPLASMOSIS

Granulocytic anaplasmosis is a tick-borne disease caused by *Anaplasma phagocytophilum* – a rickettsial pathogen, causing granulocytic blood infections in humans and animals (Alleman et al., 2008). The clinical appearance of *A. phagocytophilum* infection in dogs is defined in different ways: granulocytic ehrlichiosis (Bexfield et al., 2005) – most commonly as anaplasmosis or granulocytic anaplasmosis.

## Etiology

*A. phagocytophilum* is a Gram negative, obligate intracellular pleiomorphic agent. Up to 2001, *A. phagocytophilum* belonged to the genus *Ehrlichia phagocytophila*. This genus included *E. phagocytophila* (causative agent of the tick-borne fever in cattle, goats and sheep), *Ehrlichia equi* (causing granulocytic ehrlichiosis in horses) and an unnamed agent of human granulocytic ehrlichiosis (HGE) (Lillienook et al., 1998). On the basis of sequential analysis of 16S rRNA and groES1 operons, these three aetiological agents were united in one species and renamed to *A. phagocytophilum*. Target cells for *A. phagocytophilum* are neutrophil leukocytes and sometimes eosinophils (Pusterla et al., 1999). The earliest time when anaplasmae could be seen is 4 to 18 days after the infection as elementary bodies of 0 to 6 µm or morules of 4 to 6 µm size in the cytoplasm of blood neutrophils. Microscopically, morules could be registered for a short period of time, usually for 4 to 8 days (Egenvall et al., 1998).

## Epidemiology

Granulocytic anaplasmosis in dogs is sporadically

detected in different European countries: Sweden (Egenvall et al., 1996, 1998); Norway (Ekerstad et al., 1996); Switzerland (Pusterla et al., 1997); Italy (Gravino et al., 1997); Austria (Kirtz., 2000); Great Britain (Bexfield., 2005); Slovenia (Tozon et al., 2003); Germany (Jensen et al., 2007); and Czech Republic (Melter et al., 2007). In the USA, the disease (neutrophilic ehrlichiosis) was detected for the first time in 1971 in a German shepherd dog from Arkansas (Ewing et al., 1997). In 1998 it was also studied in 6 dogs from North Carolina and Virginia (Golzman et al., 1998). The latest data showed a remarkable wide prevalence – it was found in all states except for Mississippi and Nebraska (Alleman, 2007). *A. phagocytophilum* was evidenced for the first time in Australia in 2001, whereas the first incidence in Canada was in 2005 (Lester et al., 2005). Granulocytic anaplasmosis is a vector borne disease. The commonest vector of *A. phagocytophilum* in Europe is the tick *I. ricinus*. In 1995 it was identified as a disease vector for the first time in Sweden (Johansson et al., 1995), and then in Switzerland (Pusterla et al., 1999). The vectors in America are the ticks *I. scapularis* and *I. pasificus* (Davoust and Wamsley, 2008).

*A. phagocytophilum* is characterized with a trans-stage transmission. An infection with *A. phagocytophilum* could occur through blood transfusion (Ewing et al., 1997). There is no sex-related predisposition in dogs with regard to granulocytic anaplasmosis. Pets are not a real source of infection for humans, but are sentinel animals. Such are also cats, among which the disease is also encountered. The seroprevalence in cats varies from 4.3% to 38% (Billeter et al., 2007).

## Clinical signs

The symptoms of granulocytic ehrlichiosis are not specific – fever (up to 41°C), anorexia, lethargy, depression, vomiting, diarrhea, polyarthritis, splenomegaly, lymphadenopathy and anemia. There are cases of co-infections with *Borrelia burgdorferi* *Babesia* spp. and the tick-borne encephalitis virus (Tarello, 2003). In experimental infection, the clinical manifestation is not significant (Cohn, 2003). The described haematological deviations in this disease are: leukopenia, rarely leukocytosis, normocytic normochromic anaemia, eosinopenia and mild thrombocytopenia. At the onset of disease, thrombocytopenia may be more prominent.

## Diagnosis

The detection of *A. phagocytophilum* morules in granulocytes is a sufficient prerequisite to identify the disease (Pusterla et al. 1997). This is evidenced in 5% to 37% infected granulocytes in dogs with clinical signs of *A. phagocytophilum* infection. For a more reliable diagnosis however, the performance of additional analyses is

advised (Sirigireddy and Ganta, 2005), such as indirect immunofluorescence, PCR and isolation. Until now, all isolated strains have been cultivated on the human cell line HL-60 (Goodman et al., 1996). Since 2006, the ELISA SNAP 4Dx test kit appeared on the market (IDEXX Laboratories, Westbrook, Maine, USA), that has a high sensitivity (99.4%) and specificity (100%). It detects IgM and IgG antibodies against *A. phagocytophilum*

### Treatment

The most efficient anti-Anaplasmatic preparation is doxycycline at a daily dose of 10 mg/kg for 3 to 4 weeks (Alleman, 2007). The clinical effect is manifested within a week after the application. The premature discontinuation of the therapy could however result in *A. phagocytophilum* carrier stage.

### HEPATOZOONOSIS

Hepatozoonosis is a tick-borne disease of wild and domestic carnivores (meat eating animals) caused by protozoan parasite, *Hepatozoon canis* that is transmitted by ticks, usually the brown dog tick, *R. sanguineus*. Its mode of transmission is unusual; the tick picks up the organism from an infected host while biting the animal. An uninfected host gets the disease by eating the tick, not from being bitten by the tick. Because of the long prepatent period of the parasite, this disease is not developed soon after tick-bites of particular tick season, but there is all possibilities of occurrence of the disease all year round.

The life cycle of the apicomplexan protozoan *Hepatozoon canis* in its natural hosts *Rhipicephalus sanguineus* (tick) and *Canis familiaris* (domestic dog) was studied in an experimental infection. Tick nymphs were fed on a naturally infected dog, or they were infected by percutaneous injection of blood. Dogs were inoculated by ingestion of adult ticks containing mature oocysts. Gamonts were in syzygy 24 hr after percutaneous injection of ticks. Early oocysts were detected 96 hr after nymph repletion, and mature oocysts in adult ticks were infective to dogs 40 days postmolt. Merogony was detected in dog bone marrow from 13 days postinoculation (PI) and included meronts containing 20-30 micromerozoites, and a second type with 2-4 macromerozoites. Monozoic cysts were observed in the spleen in conjunction with merogony. Gamontogony with infection of leukocytes by micromerozoites occurred from 26 days PI, and gamont parasitemia, which completed the life cycle, was detected 28 days PI. The length of the life cycle from nymphal attachment to parasitemia in dogs was 81 days. Increased body temperatures were evident from 16 to 27 days PI and paralleled the time of intensive bone marrow merogony. Skeletal pain and recumbency were manifested in 2 dogs (Baneth et al., 2007).

### Epidemiology

The *H. canis* among dogs is widespread. Its distribution corresponds to that of the vector tick *R. sanguineus* in continents/countries such as Africa, South Europe, South America and Asia, including the Middle East, the Pacific and Indian Ocean islands (Craig, 1984; Craig, 1990; Grevery, 1993; Baneth, 2006).

The initial studies on the seroprevalence of *H. canis* in dogs showed high to low percentage of infection in different countries such as 36% in Portugal, 17.6% in Nigeria, 2.5% in India, 2.3% in Israel and 2.1% in Thailand (Gevrey, 1993). Because of its frequency of asymptomatic course, the determination of the extent and intensity of *H. canis* infection require systemic, direct and indirect studies of this parasite in dogs.

The prevalence of *H. canis* infection in the body of dogs in the different regions is also considerably varying. Circulating *H. canis* gamonts in blood have been detected in 39% of dogs, in the rural areas of Rio de Janeiro state in Brazil (O'Dwyer et al., 2001), in 22% of dogs in Zaria, Nigeria (Ezekolli et al., 1983) and in 1.2% of dogs in Malaysia (Rajamanickam et al., 1983). Similar to other tick-borne diseases as ehrlichiosis, babesiosis, Lyme diseases etc, the contact with *H. canis* in endemic regions is much more frequent than the clinical incidence of illness. Most dogs infected with *H. canis* probably develop subclinical infections. Among the dogs surveyed for the presence of *H. canis* antibodies in Israel by Baneth et al. (1997), 33% were found seropositive but only 3% had gamonts in the blood and 1% exhibited severe clinical signs of infection. The distribution of *H. canis* is closely related to that of the definitive tick host – *R. sanguineus* (Wenyon, 1931; Baneth et al., 2001). It is a three-host tick and is considered to be one of the most prevalent species worldwide (Wenyon, 1931), adaptable to different environmental conditions and found in warm and temperate region. *H. canis* has a transphasic (trans-stage) transmission from nymph to imago. Trans-ovarial transmission has not been observed. Experimentally, ticks could be infected by percutaneous injection of gamonts, containing blood (Baneth et al., 2001).

Most cases of *H. canis* infections are detected in hot seasons, when the activity of the vector is higher. However, illnesses were observed during the cold month too, most likely during persisting infection (Baneth and Weigler, 1997). The host range of *H. canis* in carnivores is not yet elucidated, except for the domestic dog.

### Transmission

Transmission of *H. canis* takes place when dogs ingest ticks containing mature oocyst in the haemocoel. *R. sanguineus* nymphs acquire the infection after feeding on an infected dog. After transstadial transmission, the tick's adult stage is infective to dogs through oral inoculation (Baneth et al., 2001, 2007).

## Clinical signs

The infection with *H. canis* in dogs could occur in three forms: subclinical, which is probably the commonest one; acute, developing about one week before the death, and chronic with phases of clinical expression and remission (Beaufils et al., 1988). The clinical signs are very various, but non-specific.

In several studies in dogs experimentally and spontaneously infected with *H. canis*, the most frequently observed clinical signs were anaemia, emaciation and intermittent fever (Baneth, 2006). On many occasions, cachexia, depression, muscle hyperaesthesia, purulent conjunctivitis and rhinitis were reported. Less frequently, diarrhea (often bloody), anorexia, paraparesis and parapalysis were observed. The low parasitaemia with gamont in less than 5% of neutrophils is the most commonly encountered extent of infection. It is generally related to asymptomatic or mild illness. The severe clinical signs are characteristics for high parasitaemia reaching 100% and often are associated with marked leukocytosis (Baneth, 2006).

## Pathology

The principal gross pathology in dogs infected with *H. canis* is cachexia. Muscle atrophy is most frequently and most visible in the temporal region. Also, anaemia, midly icteric mucous coats and slightly enlarged spleen and liver were observed. Congestive changes in the lungs and the gastric mucous coat, and pale kidneys are also communicated (Craig, 1984, 1990; Gevrey, 1993; Baneth, 2006).

Histologically, schizonts are observed in the skeletal and cardiac muscles, lymph nodes, the spleen, liver, kidneys etc. (Craig, 1984, 1990; Gevrey, 1993; Baneth, 2006). Two types of schizonts are detected: microschantons containing micromerozoites that are larger and macroschantons filled with macromerozoites. Microschantons in the various organs are observed more frequently and at higher extent at the time of schizont formation reaction. However, at the time of merozoites release, an intensive cell response is detected, consisting of equal amounts of macrophages and neutrophils and a varying number of eosinophils.

## Diagnosis

### Clinical

Anaemia is the commonest, primary haematological sign observed in most cases (Baneth, 2006). Usually, it is normocytic, normochromic and regenerative in particular. The leukocyte counts are often within the normal range, when the parasitaemia is low and increase in highly parasitaemic animals (up to 150,000/ $\mu$ l). One third of dogs,

infected with *H. canis* exhibited thrombocytopaenia, but in some instances, it is connected to co-infections such as ehrlichiosis. The changes in some serum biochemical parameters are clearly manifested and include hyperglobulinaemia and hypoalbuminaemia, increased creatine kinase and alkaline phosphatase activities (Baneth, 1997).

### Parasitological

The microscopic detection of *H. canis* gamonts in blood smears stained according to Romanovski-Giemsa, Pappenheim or with Hemacolor is the commonest diagnostic approach to this infection. The protozoa concentration is directly related to the severity of the illness. Gamonts are oval shape with dimensions of 8-12/3-6  $\mu$ m and are detected in the cytoplasm of neutrophils and rarely in that of monocytes (Baneth, 2006). Schizonts of *H. canis* could be observed in histological or touch impression preparations from lymph nodes, spleen, and bone marrow. Schizonts are round or oval, with a diameter of about 30  $\mu$ m and contain 2 or 4 macromerozoites or over 20 micromerozoites. Histologically, microschantons with the so-called "wheel spoke" shape could be observed (Baneth, 2006).

### Serological

For sero-diagnostic purposes, indirect immunofluorescent antibody test (IFAT) and ELISA are applied. Previously prepared antigen of *H. canis* gamonts is used. These assays are performed mainly in epidemiological studies (Baneth et al., 2002).

### Treatment

The primary drug used nowadays in the treatment of canine *H. canis*, is combination therapy of imidocarb dipropionate and tetracycline hydrochloride has been shown to achieve clinical cure. It is administered at 5-6 mg/kg, subcutaneously or intramuscularly at 14-day interval until disappearance of gamonts in blood. However, because of very slow elimination of gamonts in the peripheral blood, in certain cases, imidocarb dipropionate have to be administered over eight week.

Apart from this, antiprotozoal regimen, supportive care with non-steroidal anti-inflammatory drugs is very important. Some dogs with undiagnosed hepatozoonosis may recover as a result of good care by owners (Ewing and Panciera, 2003).

## BABESIOSIS

Babesiosis, a tick-borne protozoan disease of animals caused by the parasite of the genus *Babesia* is of

worldwide importance infection in dogs. It may occur by tick transmission, direct transmission via blood transfer from dog bites, blood transfusion, or transplacental transmission. The most common mode of transmission is by tick bite, the *Babesia canis* uses the tick as a vector to reach host mammals. Once infected, the *Babesia* organisms multiply within the erythrocytes of the host (Irwin, 2005).

Canine babesiosis ranges in severity from relatively mild to fatal and haemolytic anemia is the main clinical sign. Many species of *Babesia* belonging to the two forms of *Babesia* (larger form *B. canis* measuring 3-5 µm and smaller form, *B. gibsoni* measuring 1-3 µm) are pathogenic to the dog. The major *B. canis* are both host and vector specific; thus *B. canis* (subtype *vogeli*) is found exclusively in the dog with the tick *R. sanguineus* as its major vector (Soulsby 1982). *B. gibsoni* occurs mainly in Asia, North America and North and East Africa (Taboada, 1998). The common brown dog tick, *R. sanguineus* is the most predominant dog ticks in Nigeria. It does not readily attack humans but usually prefers non-human hosts for completion of its development (Okoli et al., 2006).

Canine babesiosis is endemic in Nigeria and according to Dipeolu (1975), tick vectors of *B. canis* occur in large numbers in most parts of Nigeria. The prevalence of babesiosis varies from one part of the country to another as Oduye and Dipeolu (1976) noted from blood smears of 500 dogs in Ibadan, Nigeria, that *B. canis* accounted for 53% parasitemia. Odewunmi and Uzoukwu (1979) while investigating the prevalence of blood parasites in 116 dogs at Enugu found out that *Babesia canis* accounted for 55.1%. *B. canis* can affect dogs of all ages, although young dogs are mostly affected (Lobetti, 1998).

## Etiology

*Babesia* (Apicomplexa: Piroplasmida) species are tick-transmitted parasites infecting a wide range of wild and domestic vertebrate hosts (Kuttler, 1988). Traditionally, identification of species has been based on host specificity and morphology of the intraerythrocytic piroplasms. Based on these, canine babesiae have been originally recognised to belong to two distinct species, the large pyriform (4-5 µm) *Babesia canis* and the small usually pleomorph (1-2.5 µm) *Babesia gibsoni*. On the basis of differences in geographical distribution, vector specificity and antigenic properties (Uilenberg et al., 1989; Hauschild et al., 1995), *B. canis* has been subdivided into three subspecies, namely *B. canis canis* transmitted by *D. reticulatus* and *R. sanguineus* in Europe, *B. canis vogeli* transmitted by *R. sanguineus* in tropical and subtropical regions and *B. canis rossi* transmitted by *Haemaphysalis leachi* in South Africa. These subspecies also differ from each other in pathogenicity. *B. canis rossi* causes a frequently fatal infection in domestic dogs, even after treatment; *B. canis vogeli* causes a moderate often clinically unapparent

infection; and *B. canis canis* infections result in a more variable pathogenicity intermediate between *B. canis rossi* and *B. canis vogeli* (Uilenberg et al., 1989). Genetic differences between these subspecies have also been proved by Zahler et al. (1998).

Some authors (Zahler et al., 2004) suggested and some others (Passos et al., 2005) even used the species level for these three subspecies. Baneth et al. (2004) described a fourth subspecies with unknown vector named *B. canis presentii* which has been detected in cats. Recently, a novel large *Babesia* sp. has been detected in an infected dog from North America by Birkenheuer et al. (2004) which either represents a new *Babesia* sp. or is one of the nearly 100 *Babesia* spp described for which no genetic data have been reported. Canine babesiosis caused by *B. canis* has been reported in several European countries, particularly in the Mediterranean region, where *R. sanguineus* and *D. reticulatus* are its vectors.

Cases of autochthonous large *Babesia* infections have been reported from Belgium, Croatia, France, Germany, Hungary, Italy, Poland, Slovenia, Spain and the Netherlands (Losson et al., 1999; Beugnet, 2002; Cacciò et al., 2002; Duh et al., 2004). Recent studies using molecular methods showed that in France, Slovenia and Spain where these vector species coexist, both *B. canis canis* and *B. canis vogeli* could be detected (Cacciò et al., 2002; Criado-Fornelio et al., 2003; Duh et al., 2004).

## Epidemiology

The distribution of canine *Babesia* parasites is worldwide 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., 2008), whereas *Babesia canis canis* transmitted by *Dermacentor reticulatus* is distributed mainly in Europe (Cacciò 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 Matjila et al., 2007). *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. 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).

### 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 (Solano-Gallego et al., 2008). In terms of severity of infections associated with the subspecies of *B. canis*, evidence shows that *B. canis rossii* is the most virulent with haemolytic and inflammatory responses (Reyers et al., 1998), *B. canis canis* shows a transient parasitaemia (<1%) associated with congestion of internal organs (Schetters et al., 1997), whereas *B. 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 *B. gibsoni* are usually associated with splenomegally, hepatomegally, haemolytic anaemia and severe thrombocytopenia. Clinical signs of *B. 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). *B. (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.

### 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), 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. 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 (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., 2004; Song et al., 2004). 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, per acute and chronic infections most especially in immuno compromised and young. 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 (Birkenheuer et al., 2001). Recent advances in molecular techniques have seen an avalanche of methods used to diagnose these parasites.

### Transmission

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. (2004) 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. Trans-placental 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.

### Treatment

Drugs that have been used for the treatment of canine babesiosis (Atavaquone, azithromycin, diminazene aceturate, phenamidine isethionate, pentamidine, parvaquone, niridazole 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/have the capacity to treat infection due to *Babesia canis*, the report of Birkenheuer et al. (2004) 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. Although research has shown that treatment of canine babesiosis due to *B. gibsoni* with diminazene and or imidocarb is ineffective (Birkenheuer et al., 1999), it is imperative to state that Imidocarb has the capacity to stop the multiplication of the intraerythrocytic 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*. 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 diarrhea 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 (Brandao et al., 2003).

### GENERAL PREVENTION OF TICK- BORNE DISEASES IN DOGS

1. Numerous products and medications to prevent ticks on dogs are available both over the counter and from veterinarians. Some veterinarians suggest tick collar and preventive vaccination. Although no method offers 100% protection (Baneth, 2006).
2. Stray dogs are especially vulnerable to tick-borne

diseases because of time spent in tick infested environments. Owners should therefore be diligent about applying topical or systemic tick-control treatments before letting them out (Baneth, 2006).

3. Dogs should be checked for ticks daily; if ticks are found, consider taking them to a veterinary clinic where a veterinarian or technician can remove them safely and show the dog owners how the removal is been done. Prompt removal of ticks is very essential avoids infection with pathogens (Shaw et al., 2001).
4. Avoidance of tick bites is the most cornerstone of prevention. Protective clothing (e.g., long-sleeved shirts and trousers tucked into socks) and footwear should be used in tick habitats. Ticks may be more visible on light-colored apparel. Clothing can be impregnated with acaricides such as permethrin. Tick repellents such as DEET are also helpful, although it should be kept in mind that they are only effective for a short period against ticks. For example, most tick repellents last less than 2 hours against *Amblyomma* spp. ticks. Clothing should be removed and decontaminated immediately after leaving a tick-infested environment.

### CONCLUSION

Tick-borne diseases (TBDs), including emerging and re-emerging infectious diseases, are important threats to human and animal health worldwide. Indeed, the number of reported human and animal infectious cases of novel TBD agents has increased in recent decades. However, TBDs tend to be neglected, especially in resource limited countries that often have limited diagnostic capacity (Yonqin et al., 2018).

In a study conducted in India it was discovered that on microscopy examination, only Hepatozoon gamonts were observed in twelve out of 525 (2.3%; 95% CI: 1.2, 4) blood smears. Using polymerase chain reaction (PCR), a total of 261 from 525 dogs (49.7%; 95% CI: 45.4, 54.1) in this study were infected with one or more canine tick-borne pathogen. Hepatozoon canis (30%; 95% CI: 26.0, 34.0) was the most common TBD pathogen found infecting dogs in India followed by Ehrlichia canis (20.6%; 95% CI: 17.2, 24.3), Mycoplasma haemocanis (12.2%; 95% CI: 9.5, 15.3), Anaplasma platys (6.5%; 95% CI: 4.5, 8.9), Babesia vogeli (5.5%, 95% CI: 3.7, 7.8) and Babesia gibsoni (0.2%, 95% CI: 0.01, 1.06). Concurrent infection with more than one TBD pathogen occurred in 39% of cases. Potential tick vectors, Rhipicephalus (most commonly) and/or Haemaphysalis ticks were found on 278 (53%) of dogs examined (Abd Rani et al., 2011).

Thus, the urgent need for more work to be done in developing regions of the globe to curb the devastating menace of TBDs. A more robust Molecular approach is required for the diagnosis of the species responsible for the TBDs other than the present microscopic method of diagnosis.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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