AN OVERVIEW ON CANINE BABESIOSIS

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INTRODUCTION:

The *Babesia* species belongs to the super kingdom eukaryotes, kingdom Protista, phylum Protozoa, subphylum Apicomplexa, class *sporozoea*, order piroplasmida, family *Babesiidae* and Genus *Babesia*. The family consists of several species and in that four species have been described as important in dogs and cats were *Babesia canis*, *Babesia gibsoni*, *Babesia vogeli* and *Babesia felis* (Soulsby, 1982). The relatively larger forms referred to as *B. canis* and a smaller parasite, *B. gibsoni*.The larger forms of *Babesia* spp. include *B. canis*, *B. canis rossi* and *B. canis vogeli*.With regard to the smaller piroplasms, three genetically and clinically distinct species are currently recognized causing the disease in dogs. They are *B. gibsoni*, *B. conradae* and a *B. microti* like piroplasm (named *Theileria annae*). *Babesia gibsoni* has a worldwide distribution and is transmitted by *Haemaphysalis* spp. with a variable degree of virulence (Augustine, 2013).*Babesia gibsoni*was first reported in hounds and jackals in India and since then it was recognized in Asia, America, northern and eastern Africa and rarely in Europe (Zahler *et al*., 2000). *Babesia gibsoni*, a smaller form of parasite causing canine babesiosis was reported principally in the Middle East, Southern Asia, Japan, North Africa and South America, and considered as an emerging infection in the United States of America as well as having been detected later in Australia, Hungary and Italy (Muhlnickel *et al*., 2002).

Kerala

Canine babesiosis due to *B. canis* and *B. gibsoni* were first reported from Thrissur in Kerala (Sabu *et al*., 2002; Sabu, 2005) followed by Karunakaran *et al*. (2011) in a German shepherd dog from Palakkad district in Kerala. Later, Augustine (2013) reported that 30 (25.86 per cent)and four (3.45 per cent) out of 116 dogs were found to be infected with *B. gibsoni* and *Babesia canis* respectively in a study on morphological and molecular detection of *Babesia* spp. in dogs in Thrissur. Tresamol *et al*. (2013) documented a case report of cerebral babesiosis due to *B. gibsoni* infection in a Boxer dog from Thrissur district in Kerala.

EPIDEMIOLOGY

The young dogs were highly susceptible to babesiosis and most frequently had severe infections and suffered from acute and hyper acute forms than the older dogs (Muhlnickel *et al*., 2002; Boozer and Macintire, 2005). *B. canis* was observed in 36 hours old puppies and *B. gibsoni* in three day old puppies and concluded transmission of piroplasms by transplacental route because the age was shorter than the prepatent period.The incidence of canine

babesiosis was 10.6 times higher in less than two years of age when compared to older dogs while older dogs were predisposed to more complications when compared to younger ones (Bashir *et al*., 2009).Dogs screened for haemoprotozoal infections were positive for *B. gibsoni*, among them 58 per cent of neonate population were less than three month old and 18 per cent were less than one year old (Selvaraj *et al*. 2010). *B. gibsoni* was found to be higher among dogs less than two years of age (Augustine, 2013). Canine babesiosis was most common in male dogs when compared to female dogs due to behavioural activities like roaming, searching for mates and establishment of territories that resulted in them getting infested with ticks (Amuta *et al*., 2009; Bashir *et al.* 2009). Majority of dogs diagnosed with *B. gibsoni* in the United States were American Staffordshire terriers and American Pit Bull Terriers (APBT) (Birkenheuer *et al*. 2003). Babesiosis in dogs from northern Portugal occurred during auntum and winter months *viz.,* October (18 per cent), November (27per cent), December (20 per cent), February (13per cent) and March (9 per cent) (Cardoso *et al*. (2008). canine babesiosis was more prevalent during the months of January, July, August, September and November compared to February, March, April, May, October and December in a detailed epidemiological and vector identification study in Pakistan (Bashir *et al.* 2009).

TRANSMISSION

Babesia gibsoni infection in dogs was transmitted by ticks *Rhipicephalus sanguineus* and *Haemaphysalis bispinosa* (Taboada and Lobetti, 2006; Aysul *et al*., 2013; Augustine 2013). Other mode of transmission included transplacental transmission, the direct transmission of blood during dog bites, direct transmission of blood by iatrogenic means and shipping of dogs from endemic areas (Macintire *et al*., 2002).

PATHOGENESIS

Pathophysiology of canine babesiosis varied from mild anaemia to widespread multiple organ failure and death

The anaemia caused by *B. gibsoni* was due to destruction of erythrocytes, resulted from a combination of the direct mechanical disruption caused by the parasite as it leaves the red blood cell, along with intravascular haemolysis, which may be immune mediated or non-immune mediated destruction of erythrocytes. The indirect pathways of RBC destruction included immune mediated destruction secondary to the development of antierythrocytic membrane antibodies, inhibition of erythrocyte 5' nucleosidase, development of methemoglobinemia secondary to oxidative stress, induction of serum haemolytic proteins and increased macrophage erythrophgocytic activity. Oxidative damage of erythrocytes induced by *B. gibsoni* infection, even in the presence of low parasitaemia led to severe anaemia (Otsuka *et al*., 2002). Significant increase in the level of anti-erythrocyte membrane antibodies (IgG andIgM) in sera of dogs naturally infected with *B. gibsoni*. However the antibodies were suggested as possible enhancers of erythrocyte destruction and this was confirmed by ELISA and immunoblotting technique. Immunosuppression was observed in dogs suffered from relapses of clinical *B. gibsoni* infection and it could be due to prominent depression of lymphocyte blastogenesis and anti-parasitic antibody production (Adachi *et al*., 1993). Dogs experimentally infected with *B. gibsoni* had anatomic lesions *viz.,*diffuse nonsuppurative periportal hepatitis, centrilobular hepatitis, multifocal necrotizing arteritis, membranoproliferative glomerulonephritis, reactive lymphadenopathy, diffuse erythrophagocytosis, and extramedullary haematopoiesis (Wozniak *et al*., 1997).

Infection with *B. gibsoni* resulted in more severe clinical manifestations with multiple organ failure than infection with *B. canis* in Japan hence infection caused by *B. gibsoni* was considered clinically more important than *B. canis* (Miyama *et al*. 2005). Anaemia in *B. gibsoni* infection might be due to direct parasite- induced red-cell damage, increased osmotic fragility of infected red blood cells, oxidative and secondary immune-mediated injury of the erythrocyte membrane resulting in a combination of intravascular and extravascular haemolysis (Irwin, 2009).

SEVERE OR COMPLICATED BABESIOSIS:

Acute renal failure, hepatopathy, coagulopathy, secondary immune mediated haemolytic anaemia, haemoconcentration, hypotension, cardiac related alterations, acute pancreatitis and acid base disturbances were reported in complicated or severe form of babesiosis. Cerebral babesiosis characterized by combination of incoordination, pelvic limb paresis, muscle tremors, nystagmus, anisocoria, intermittent loss of consciousness, seizures, stupor, coma, aggression, paddling, or vocalization (Birkenheuer, 2012). Multi-system disease and multiple organ dysfunction syndromes (MODS) developed from Systemic Inflammatory Response Syndrome (SIRS), were responsible for complicated cases of canine babesiosis by *Babesia canis*. Systemic Inflammatory Response Syndrome (SIRS) was characterized by uncontrolled inflammatory response with one or more organ dysfunction including cerebral, acute renal, hepatic dysfunction, rhabdomyolysis, adult respiratory distress syndrome, pancreatitis, dermal necrosis, haemorrhagic diathesis and secondary immune mediated haemolytic anaemia. Shock in babesiosis resulted from severe anaemia or release of inflammatory mediators associated with MODS (Jacobson and Clark, 1994; Matijatko *et al*., 2010). Acute Respiratory Distress Syndrome (ARDS) was common and an important complication of the more pathogenic strains of *Babesia* spp. characterized by tachypnoea, dyspnoea, a moist cough, serosanguinous frothy respiratory secretions and hypoxemia. Radiographs revealed either diffuse or caudo dorsal patchy alveolar infiltrate with normal cardiac silhouette and vessel size (Ayoob *et al.*2010).

CLINICAL SIGNS

Acute form of canine babesiosis was characterized by pyrexia, weakness, pallor of mucous membranes, depression, lymphadenopathy, splenomegaly and general malaise (Muhlnickel *et al.*, 2002; Jefferies *et al*., 2007). The fatal form of disease exhibited some signs like melena and local erythema of skin. Bleeding from vein puncture site was attributed to thrombocytopenia and coagulopathy which was the major presenting clinical sign and complicating factor in more number of cases (Johan Schoeman and Leisewitz 2006; Selvaraj *et al*. 2010). Intermittent vomiting also reported in a *B. gibsoni* infected dog from Italy (Trotta *et al*. 2009). Wide range of clinical presentations from subclinical disease to serious illness characterised by fever, pallor, jaundice, splenomegaly, weakness, systemic inflammation, hyperglobulinemia, neutrophilia with left shift,thrombocytopenia and pigmenturia in canine babesiosis (Jacobson *et al*. 2006).

Chronic form of *B. gibsoni* infection was manifested as a subclinical infection or associated with weight loss and weaknes (Solano-Gallego and Baneth, 2011). Clinical signs of cerebral babesiosis due to *B. gibsoni* in a dog consisted of anorexia, weakness, ataxia, occasional seizures and depression (Tresamol *et al*. 2013). Dogs experimentally infected with *B. canis* were characterized by fever, increase in pulse, tachycardia, anorexia, lethargy, pallor of the mucous membrane of the mouth and

eye, emaciation, muscle tremor, respiratory distress, nervousness, drooling salivation, haemoglobinuria, mucoid ocular discharge and followed by death if not treated (Konto *et al*. 2014). Few cases of canine babesiosis caused by *B. gibsoni* were complicated with multi-organ failure, hepatopathy, acute renal failure, immune-mediated haemolytic anaemia and cerebral babesiosis (Vijayalakshmi *et al*. 2014). Rarely cutaneous lesions manifested as oral or cutaneous petechial and ecchymotic hemorrhages associated with thrombocytopenia or disseminated intravascular coagulation, subadjacentl eukocytoclastic vasculitis with or without vascular necrosis. Clinical signs include edema, ecchymosis, ulceration, and necrosis, which can be seen on the pinnae, axillae, groin, lower limbs, ear tips or scrotum. (Miller *et al*. 2013).

DIAGNOSIS

Three fundamental techniques for diagnosis incorporates microscopic examination, serological testing and nucleic acid based detection by molecular methods

Single to multiple, variable sized (1 - 3µm in diameter), and round to oval to band like piroplasms within many red blood cells consistent with small form of *Babesia spp* in Wright's staining technique (Trotta *et al*. 2009). *Babesia gibsoni* piroplasms appeared highly pleomorphic and exhibiting different forms such as linear, amoeboid, reticulate, paired pyriform, and signet ring form (Armando *et al*., 2001). These piroplasms were pleomorphic and exhibited linear, reticulate (network forming), pyriform, amoeboid, and signet ring forms and the latter was reported as the most common form (Fukumoto *et al*., 2000;Augustine 2013). *Babesia* parasites were usually visualized in blood smears only during the acute phase and the reason for lower percentage of *Babesia* positive cases by direct examination of blood smears was attributed to less number of infected cells in peripheral blood (M'ghirbi and Bouattour 2008). Indirect fluorescent antibody test is the most regularly utilized test for identification of anti-babesial antibodies. Ano *et al*. (2001) conducted the molecular survey of *Babesia*s pp. infection among dogs in Japan and developed a new method of nested PCR. Seminested PCR for the diagnosis of canine babesiosis which differentiated *B. gibsoni* (Asian genotype), *B.canisvogeli*, *B.caniscanis* and *B.canisrossi* with defined limit of detection. Outer primer pairs 455-479F, 793-772R and species specific primers BgibAsia-F, was used in first and second round of PCR respectively to successfully amplify a 185bp products of *B. gibsoni* (Asian genotype) (Birkenheuer *et al*. 2003). Multiplex PCR can be utilized to at the same time distinguish potential co-infection with numerous tick-borne pathogens including *Babesia* spp.

TREATMENT

Diminzeneaceturate for the treatment of both small and large *Babesia* spp. infections should be used with caution account of a relatively small dose safety margin with a large inter-individual pharmokinetic variation (Miller *et al*., 2005).

Wulansari *et al*. (2003) reported that clindamycin, a dose dependant antibiotic with the property of immune enhancing ability inactivated or damaged *B. gibsoni* organisms in infected dogs. Combination therapy of clindamycin, doxycycline and metronidazole showed a rapid recovery from anaemia and thrombocytopenia or a long disease-free period compared to the untreated control dogs (Suzuki *et al*. 2007).

Atovaquone (13.3 mg/kg body weight, PO, q8h and azithromycin (10 mg/kg body weight, PO, q24h hours) for 10 days could eliminate chronic *B. gibsoni* (Asian genotype) infections or suppress the parasitemia below

the limit of detection in majority of the treated dogs (Birkenheur *et al*. 2004). Atovaquone, a hydroxynaphthoquinone acts primarily to inhibit the parasite's mitochondrial electron transport chain, possibly by mimicking the natural substrate ubiquinone. Resistance to atovaquone was rapid and resulted from a single point mutation in the gene for cytochrome b (Rang *et al*., 2012).

PREVENTION:

Doxycycline @ 10 mg /kg body weight BID for eleven consecutive days was effective in preventing babesiosis due to *B. gibsoni* but infection could not be cleared by the same (Vercammen *et al*., 1996).

Soluble parasitic antigen (SPA) from plasma of *Babesia canis* infected animals or supernatant of in vitro cultures of these parasites could be used as vaccine. The antigen treated with formalin and freeze dried when used as a vaccine against *B. canis* in dogs could decrease the incidence of babesiosis from 16 per cent to almost zero in vaccinated dogs (Schetters, 2005). A preliminary study on the safety of a new vaccine against canine babesiosis containing *B. canis* soluble parasitic antigen (SPA) and reported that the vaccine stimulated the development of antibodies prevented the development of the disease. It was found to be considered to be safe without any adverse effects (Adaszek, *et al*. 2012). Two vaccines were used against canine babesiosis *viz.*NobivacPiro® (NP) and Pirodog® (P) in France (Freyburger *et al*. 2011).

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