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Review Article

Complications and multiple organ dysfunctions in canine babesiosis: A review

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Abstract

Canine babesiosis is a clinically significant haemoprotozoan disease of domestic and wild canids caused by different *Babesia* species. It is one of the most important tick-borne infections of dogs extending globally. This review provides a thorough overview of the literature pertaining to the geographical distribution, life cycle as well as transmission of *Babesia* species affecting dogs. It congregates most of the information published in peer-reviewed journals to highlight the complications and multiple organ dysfunctions together with recent advances in understanding the pathophysiology of the disease.

Keywords: *Babesia,* Clinical manifestation, Complication, Dog, Multiple organ dysfunction

Highlights

- With its constantly shifting distribution, canine babesiosis is becoming a major global concern.
- Canine babesiosis may cause multiple organ failure and eventually, death.
- Treatment for *Babesia* spp. may not necessarily resolve the pathological changes in organs induced by the parasite, thereby complicating the therapeutic approach.
- Scientific evidence regarding the effectiveness of drugs employed in the treatment of canine babesiosis caused by small *Babesia* spp. is notably insufficient.
- Understanding the pathogenesis of multiple organ involvement in canine babesiosis is helpful in the development of improved novel treatments.

INTRODUCTION

Babesiosis is one of the most common tick-borne haemoprotozoan infection of animals and humans throughout the world (Skrabalo and Deanovic, 1957; Schnittger *et al*., 2012; Beck *et al*., 2017). It is caused by haemotropic protozoa of the genus *Babesia*, family Babesiidae, order Piroplasmida and phylum Apicomplexa (Vial and Gorenflot, 2006). It has a significant negative impact on the health of livestock and human as well as on the global economy (Homer *et al*., 2000; Jacob *et al*., 2020; Kumar *et al*., 2021). It is a significant disease of dogs, cattle and horses and is an emerging zoonoses (Vial and Gorenflot, 2006). *Babesia* is also named "Piroplasm" due to their pear-shaped appearance within erythrocytes seen under light microscopy (Petra *et al*., 2018). There are many similarities between babesiosis and human malaria regarding disease manifestations like fever, haemolysis and haemoglobinuria, and immune responses (Warren *et al*., 2015; Djokic *et al*., 2021). Therefore, sometimes acute babesiosis is named as "animal malaria" (Jacobson and Clark, 1994; Galan *et al*., 2018) and human babesiosis is even named "Malaria of the North" (Klevtsova *et al*., 2015).

Canine babesiosis is one of the most significant tick-borne haemoprotozoan disease (Yao *et al*., 2014) of domestic and wild canids occurs worldwide (Irwin, 2010; Williams *et al*., 2014; Mierzejewska *et al*., 2021). The clinical picture of canine babesiosis ranges from subclinical infection to impairment of many internal organs and even death (Yao *et al*., 2014). Loss of appetite, pyrexia, pale mucous membrane, anaemia, depression, icterus and enlarged spleen are the characteristic symptoms (Boozer and Macintire, 2003; Zygner *et al*., 2014; Kules *et al*., 2014; Beugnet *et al*., 2019). The infection may lead to injury of many internal organs and may result in systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) (Zygner *et al*., 2021). Marked haemolytic anaemia, severe acid-base abnormalities, complications such as hepatopathy with evident icterus, hypoglycaemia, acute renal failure, and failure of multiple organs occur in severe form (Keller et *al*., 2004). Identification of the *Babesia* spp.

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is utmost important for treatment. Therefore, for an accurate diagnosis of *Babesia* parasite, an integrative approach is recommended, which includes clinical picture, blood smears examination by microscopy, serological, molecular and mass spectrometry-based diagnosis. Treatments of canine babesiosis consist of antiprotozoal drugs to remove the parasite, transfusion of blood in severe anaemia, and supportive treatment for the complications and metabolic derangement associated with the disease. Specific therapy against *Babesia* spp. may or may not heal the pathological alterations in organs caused by the parasite, which makes the line of treatment more challenging. Supportive therapy should be instituted based on thorough assessment of patient for involvement of the organ. This review focuses specially on complications and MODS associated with canine babesiosis.

Historical background of discovery and nomenclature of *Babesia* spp.

The initial known reference to babesiosis is thought to be in the biblical book of Exodus 9:3, where it is referred to as a plague affecting the cattle of the Egyptian Pharaoh Ramses II, perhaps it might have been the case of red water fever as the haemoglobinuria was prevalent sign (Vial and Gorenflot, 2006). But the genus *Babesia* was named after Victor Babes, a Romanian bacteriologist, who originally described micro-organism in the erythrocytes of Romanian cattle with red water fever (hemoglobinuric fever) in 1988 (Babes, 1888). Then it was suspected to be a bacterium rather than a protozoon. Shortly thereafter, he described similar pathogen in the erythrocytes of sheep (Babes, 1892). In 1893, the causative agent of Texas cattle fever was discovered by Smith and Kilborne (1893), and they named the pathogen *Pyrosum bigemina*. They discovered that the tick was the vector for transmission of Texas cattle fever, and it was the first report of an arthropod-transmitted protozoan parasite. Later, the parasite described by Babes, and Smith and Kilbourne were named *Babesia bovis, B. ovis and B. bigemina*, respectively (Starcovici, 1893; Mihalca *et al*., 2010). Subsequently, *Babesia* parasitizing erythrocytes of dog and horse was observed and named *B. canis* (Piana and Galli-Valerio, 1895) and *B. caballi* (Koch, 1904), respectively. Wilson and Chowning (1979) noted intraerythrocytic parasites in fresh blood smear of the human patient suffering from spotted fever and named the causative agent *Pyroplasma hominis* and the diseases *Pyroplasmosis hominis*. With the advancement in microscopy, cell biology and molecular techniques, knowledge of the *Babesia* is rapidly expanding (Roncalli Amici, 2001; Uilenberg, 2006; Solano-Gallego and Baneth, 2011; Koster *et al*., 2015).

Geographical distribution of canine babesiosis

Babesia species in dogs comprise of two main species, *B. canis* (large form) and *B. gibsoni* (small form) on the basis of their size and morphologic appearance within the erythrocytes (Purnell, 1981). *Babesia gibsoni* forms small round to oval piroplasms (intraerythrocytic merozoites measure 1 to 2.5 µm), whereas *B. canis* forms large pear-shaped piroplasms (merozoites measure 3 to 5 µm) that usually occur in pairs within the erythrocyte (Meinkoth *et al*., 2002; Boozer and Macintire, 2003; Singh et *al*., 2014; Karasova *et al*., 2022). Earlier, all large *Babesia* were designated *B. canis,* while all small *Babesia* were considered to be *B. gibsoni* (Boozer and Macintire, 2003; Koster *et al*., 2015; Baneth, 2018). *Babesia* species of dogs with their transmitting vector and geographical distribution have been mentioned in Table 1. Based on geographical distribution, antigenic properties, cross-immunity, serological testing, specific

Table 1. ***Babesia*** **species of dogs with their transmitting vector and geographical distribution**

Form	Sp.	Size (µm)	Vector tick	Geographical distribution	Reference
Large form	*Babesia canis*	2 x 5	*Dermacentor reticulatus*	Central and northern Europe	Beck *et al*., 2009; Cassini *et al*., 2009; Welc-Faleciak *et al*., 2009; Oines *et al*., 2010; Imre *et al*., 2013a; Potkonjak *et al*., 2015; Solano-Gallego *et al*., 2016; Mrljak *et al*., 2017
	Babesia vogeli	2 x 5	*Rhipicephalus sanguineus*	Africa, Asia, Australia, Southern Europe, North and SouthAmerica	Birkenheuer *et al*., 2003b; Passos *et al*., 2005; Mghirbi and Bouattour, 2008; Solano-Gallego *et al*., 2008; Beck *et al*., 2009; Yisaschar-Mekuzas *et al*., 2013; Gabrielli *et al*., 2015

Cont. Table 1.

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Form	Sp.	Size (μm)	Vector tick	Geographical distribution	Reference
	Babesia rossi	2 x 5	*Haemaphysalis eliptica*	Southern and Eastern Africa	Oyamada *et al*., 2005; Penzhorn *et al*., 2017
	Babesia sp. (cocco)	2 x 6	Unknown	Eastern USA	Birkenheuer *et al*., 2004
Small form	*Babesia gibsoni*	1 x 3	*Haemaphysalis longicornis*	Asia, Africa, Australia, North America, Europe	Trapp *et al*., 2006; Beck *et al*., 2009; Tabar *et al*., 2009; Trotta *et al*., 2009; Davitkov *et al*., 2015; Solano-Gallego *et al*., 2016; Tuska-Szalay *et al*., 2021
	Babesia conradae	0.3 x 3	*Rhipicephalus sanguineus* (assumed)	America (Western USA)	Kjemtrup and Conrad, 2006; Kjemtrup *et al*., 2006; Dear *et al*., 2018
	Babesia microti-like (Theileria annae)	1 x 3	*Ixodes* spp. (assumed)	Northern Spain, other European countries, Portugal, Croatia, North America	Beck *et al*., 2009; Yeagley *et al*., 2009; Simoes *et al*., 2011; Falkeno *et al*., 2013; Gabrielli *et al*., 2015; Rene-Martellet *et al*., 2015; Miro *et al*., 2015

transmitting tick vector, pathogenicity and molecular phylogeny, *B. canis* has been further classified into three subspecies: *B. canis canis*, *B. canis vogeli* and *B. canis rossi* (Uilenberg *et al*., 1989; Schetters *et al*., 1997; Koster *et al*., 2015). Later, by molecular phylogenetic analysis, particularly targeting 18S rRNA gene, it has been determined that these subspecies are actually distinct species, namely *B. canis*, *B. vogeli* and *B. rossi* (Zahler *et al*., 1998; Uilenberg, 2006; Schnittger *et al*., 2012). Another genetically distinct (not yet named) large *Babesia* sp. (coco) related to *Babesia bigemina* has been found in immunocompromised dogs in North Carolina, New Jersey and New York, USA (Birkenheuer *et al*., 2004). Among the large forms, *Babesia rossi* is prevalent in South and East Africa (Oyamada *et al*., 2005; Penzhorn *et al*., 2017). *Babesia canis* is widespread in Asia and Europe (Karbowiak, 2014). *Babesia vogeli* has a global distribution and occurs in Asia, South Africa, Brazil, Australia, France, Japan, North America, South America, Turkey and Europe (Birkenheuer *et al*., 2003b; Passos *et al*., 2005; Mghirbi and Bouattour, 2008; Yisaschar-Mekuzas *et al*., 2013; Gabrielli *et al*., 2015). Within the small forms, three distinct species have been documented in dogs: *B. gibsoni* (previously Asian isolate), *Babesia conradae* (previously Californian isolate) and *Babesia microti*-like piroplasm (Kjemtrup and Conrad, 2006; Kjemtrup *et al*., 2006; Beck *et al*., 2009; Irwin, 2009; Koster *et al*., 2015; Crnogaj *et al*., 2017). *Babesia gibsoni* (Asian genotype) is endemic in North America, Asia, Africa, Australia and Europe (Yamane *et al*., 1994; Birkenheuer *et al*., 1999; Macintire *et al*., 2002; Solano-Gallego *et al*., 2016; Tuska-Szalay *et al*., 2021). Among small *Babesia* spp., *B. gibsoni* is considered as an emerging pathogen, and its significance is increasing in dogs in North America and Europe (Boozer and Macintire, 2005; Solano-Gallego *et al*., 2016). *Babesia conradae* is endemic in California and is closely related to isolates from wildlife and humans from the western United States (Dear *et al*., 2018). In northwest Spain, *Babesia microti*-like piroplasm is endemic in dogs (Miro *et al*., 2015). It is still not clear whether *Babesia microti*-like piroplasm is a member of *Babesia* or *Theileria* genus because it is closely related to rodent piroplasm *B. microti* and *Theileria* species (Miro *et al*., 2015). Because of this disagreement, several synonyms have been used for this, such as *Babesia*-Spanish dog isolate, *Babesia annae*, *Theileria annae*, *Babesia cf. microti*, *Babesia vulpes* (Baneth *et al*., 2015). Another piroplasm causing a disease named 'bloody ears' in dogs has been identified in Brazil (Soares *et al*., 2014).

Transmission and life cycle

Life cycle of *Babesia* is dependent on both ticks and vertebrate hosts. Life cycle is divided into three distinct stages: gametogony, sporogony and merogony with alternating asexual and sexual multiplication (Jalovecka *et al*., 2018). Asexual reproduction occurs within veterbrate's erythrocytes and sexual reproduction occurs in ticks (Koster *et al*., 2015). The

infected adult female tick is the most important vector of transmission. The sporozoites present in the saliva of tick are injected into the blood circulation of host (Taboada and Merchant, 1991; Taboada, 1998). These sporozoites get attached on erythrocytes' surfaces and then engulfed into the cytoplasm by endocytosis, where merogony occurs. The sporozoites differentiate into trophozoites, and these trophozoites multiply by binary fission into two or four merozoites. The merozoites come out of the erythrocytes by destructing them, and they invade nearby erythrocytes for the persistence of the infection in the host (Vannier *et al*., 2015). When the ticks feed on the infected host, they become infected with merozoites and may remain infective for many generations through transstadial and transovarial transmission (Taboada, 1998). Within the salivary glands of tick, sporogony occurs (merozoites multiply by multiple fission into sporozoites) (Taboada and Lobetti, 2006; Jalovecka *et al*., 2018). The sporozoites are transmitted to host circulation via saliva on bite and merogony occurs. Some merozoites transform into pre-gametocytes (gamonts) in the erythrocytes of vertebrate host. When tick feed on infected host, gamonts are ingested by tick and inside the tick's gut gametogony occurs. The male and female gametes also called as ray bodies or Strahlenkorper, fuse to form diploid zygote (Mehlhorn and Schein, 1984), which undergo meiosis and haploid kinetes are formed. These kinetes enter the haemolymph and via haemolymph reach to salivary glands and ovaries. In the tick's salivary glands, kinetes multiply and differentiate into sporozoites. Vertebrate host may become infected by sporozoites, only after the tick transforms into the next stage i.e. from larvae to nymph, from nymph to adult, called transstadial transmission. In transovarial transmission, kinetes reaches to ovary and then to eggs of female ticks and the sporozoites are formed in the salivary glands of the next generation larvae of infected ticks (Schnittger *et al*., 2012). Once the infected tick feeds on a new vertebrate host, the sporozoites mature in the tick's salivary glands in 2-3 days to become infective. Therefore, vertebrate hosts acquire the *Babesia* infection when ticks feed on them for 2 to 3 days (Taboada and Merchant, 1991; Taboada, 1998). *Babesia* spp. are mainly transmitted via the tick bite, but other non-vector routes of transmission have also been documented in dogs. *Babesia gibsoni* transmission has been documented via blood transfusion (Stegeman *et al*., 2003), transplacental transmission (Abu, 1973; Fukumoto *et al*., 2005) and vertical transmission (Solano-Gallego *et al*., 2016). Several studies have provided evidence of direct transmission of *B. gibsoni* between dogs through bite wounds, saliva, or blood ingestion (Birkenheuer *et al*., 2003a; 2005; Jefferies *et al*., 2007; Yeagley *et al*., 2009; Kirk *et al*., 2017), which typically involve the illegal "fighting" of dogs and the American Staffordshire Terrier and American Pit Bull Terrier as relevant breeds (Kirk *et al*., 2017; Birkenheuer *et al*., 2018). Direct transmission of *B. gibsoni* may also occur through the sharing of instruments for ear cropping and tail docking, and through the re-use of vaccination needles of *B. gibsoni* infected dogs (Macintire *et al*., 2002).

Factors influencing babesiosis in dogs

Clinical manifestations of canine babesiosis vary with the parasite factors (species and virulence of *Babesia*), host factors (age, individual immune status), environmental factors, vector distribution and the existence of coexisting infections (Jacobson, 2006; Irwin, 2009; Tsegay *et al*., 2016). Prevalence of babesiosis is more in dogs living in tick abundant area or had travelled to a tick endemic area (Irwin, 2010). The methods employed for diagnosis additionally impact the reported variation in prevalence (O'Dwyer *et al*., 2009; Kushwaha *et al*., 2018). The pathogenicity and occurrence of *Babesia* spp. varies from region to region, which might be due to the differences in prevailing agro-climatic conditions that favour the survival of *Babesia* spp. and their vectors (Tsegay *et al*., 2016). The incidence of babesiosis in dogs is related to the annual seasonal pattern of specific locality and seasonal activity of tick vector. It is more prevalent in monsoon months, which might be due to a greater number of ticks in monsoon (Bansal *et al*., 1985; Sharma *et al*., 2011). Singh *et al*. (2014) found that babesiosis in dogs is most prevalent in the warm season compared to winter. Most clinical cases occur in the spring and autumn season (Matijatko *et al*., 2012). The seasonal pattern of canine babesiosis in Europe has markedly changed, possibly as a result of climate change, altered land use, increase in wild animal population, spreading of vectors by wildlife (birds and animals), and altered wildlife habitat structure (Leschnik *et al*., 2008).

The clinical manifestations of babesiosis in dogs range from subclinical to fatal depending on the species of *Babesia* involved and the immune response of the host (Solano-Gallego and Baneth, 2011; Zygner *et al*., 2023). An infection caused by *B. conradae*, *B. canis* and *B. gibsoni* result in mild to severe illness (Irwin, 2009; 2010; Penzhorn, 2011; Solano-Gallego and Baneth, 2011; Karbowiak, 2014). *Babesia gibsoni* infection causes severe clinical symptoms such as

pyrexia, haemolytic anaemia, splenomegaly and lethargy; however, depending on the pathogenicity of the strain, the host's immune system or their age, subclinical infection may occur (Solano-Gallego and Baneth, 2011; Kirk *et al*., 2017).

Babesia vogeli is considered to be the least pathogenic among *Babesia* species infecting dogs and often leads to moderate or clinically inapparent infections (Uilenberg *et al*., 1989; Zahler *et al*., 1998; Caccio *et al*., 2002). In asymptomatic dogs, the higher prevalence of *B. vogeli* was observed compared to symptomatic dogs (Beck *et al*., 2009). *Babesia rossi* is most virulent (Oyamada *et al*., 2005) and often causing peracute disease instigating tissue hypoxia, hypotensive shock, multiple organ dysfunction, disseminated intravascular coagulation (DIC) and death (Barr and Bowman, 2006; Jacobson, 2006). *Babesia conradae* is more virulent (Kjemtrup *et al*., 2006) and may produce the highest level of parasitaemia with more pronounced anaemia and higher fatality rates than *B. gibsoni* (Kjemtrup and Conrad, 2006).

Adult dogs have a higher prevalence of *Babesia* spp. infection compared to puppies (Nalubamba *et al*., 2015; Tsegay *et al*., 2016; Mahalingaiah *et al*., 2017). The rise in the number of seropositive dogs with age may be associated with the prolonged duration of exposure to transmitting vectors over the years. But it does not hold true in all cases. Younger dogs affected more with babesiosis than adult dogs (Samradhni *et al*., 2005; Sahu *et al*., 2014; Singh *et al*., 2014), which may be related to underdeveloped immune system in young dogs as compared to adults (Tizard, 2004). Whereas, Kushwaha *et al*. (2016) found maximum cases of *B. gibsoni* in dogs over 4 years of age, and minimum cases were recorded in younger dogs (less than one year of age). However, comparable prevalence of *B. gibsoni* infection has been recorded in all age groups of dogs (Vipan *et al*., 2015).

Higher incidence of *Babesia* spp. infection have been recorded in male dogs than female dogs (Nalubamba *et al*., 2015; Vipan *et al*., 2015; Kushwaha *et al*., 2016; Gonde *et al*., 2017; Mahalingaiah *et al*., 2017), owing to frequent roaming behaviour of male dogs in search of mates and territory. However, slightly higher incidence of *Babesia* spp. infection was recorded in female dogs than in males (Tsegay *et al*., 2016). The reason may be that the females are exposed to many male dogs during estrus period, which exposes them to different parasites. A number of clinical cases of babesiosis have been reported in Labrador Retrievers and German shepherds compared to other breeds (Gonde *et al*., 2017; Mahalingaiah *et al*., 2017). However, despite the statistical significance, babesiosis is not connected with the predisposition of particular breeds (Singh *et al*., 2014). It is related with the housing conditions of dogs and the nature or purpose of their work (Bourdoiseau, 2006; Adaszek *et al*., 2011). It is significantly higher in hunting and outdoor dogs compared to indoor dogs (Mrljak *et al*., 2017). The survey conducted in Western Romania confirmed that hunting behaviour poses a significant risk of contracting *B. canis* infection (Imre *et al*., 2013a). *Babesia gibsoni* is more prevalent in fighting breeds of dogs in the USA (Cannon *et al*., 2016) and Europe (Imre *et al*., 2013b). Fighting dogs are particularly reservoir for *B. gibsoni,* and it is speculated that *B. gibsoni* will eventually be reported from the countries where dog fighting is practiced (Irwin, 2009).

Clinical manifestations

The clinical outcome of *Babesia* infection is determined by the grade of haemolytic anaemia and the immune response of the host against parasitaemia. Haemolytic anaemia and a systemic inflammatory response account for most of the clinical manifestations and may lead to organ dysfunction (Baneth, 2018). Erythrocyte lysis is related to broad spectrum of clinical manifestations in canine babesiosis. The involvement of haemolysis in the progression of babesiosis was illustrated through a proteomic method (Kules *et al*., 2014). The onset of the disease is typically sudden, affected dogs showing symptoms such as fever and lethargy. Following this initial phase, they may exhibit clinical signs of anaemia, as well as dysfunction in the liver, lungs, kidneys, or brain and haemostatic abnormalities including coagulation and electrolyte imbalances. Cases of sub-clinical and sub-acute infections have also been documented (Leisewitz *et al*., 2001; Jacobson, 2006; Irwin, 2009; Eichenberger *et al*., 2016). The most common clinical symptoms in canine babesiosis are apathy, weakness, anorexia, pyrexia, pale mucous membranes and poor general condition (Tarello, 2003; Gonde *et al*., 2016; 2017; Solano-Gallego *et al*., 2016). The rare findings are hind limb weakness, ascites, jaundice, paraplegia, melena, blindness, ocular bleeding, immune-mediated haemolytic anaemia (IMHA) and skin lesions (Gonde *et al*., 2017). Unlike bovine babesiosis, haemoglobinuria is infrequent in canine babesiosis (Gupta *et al*., 2002; Varshney *et al*., 2008). Anaemia, thrombocytopenia and hypoalbuminemia are the main abnormal haemato-biochemical findings in canine

babesiosis (Behera *et al*., 2015). The majority of dogs that have recovered establish a state of premunition, which represents a balance between the immune response of the host and the parasite's capacity to cause clinical illness. After initial parasitaemia, the immune system of the host is unable to fully eliminate the infection, and the host remains in a persistent carrier state. In these hosts, infection may relapse months to years later, and long-term sequelae (glomerulonephritis or polyarthritis) may develop (Lobetti, 1998).

Canine babesiosis can be clinically categorised as uncomplicated and complicated form (Jacobson and Clark, 1994; White *et al*., 2013). An uncomplicated form of babesiosis is a consequence of haemolytic anaemia (Jacobson and Clark, 1994) whereas, complicated babesiosis may be a consequence of anaemia, tissue hypoxia and inflammatory mechanisms that result in the emergence of SIRS and MODS caused by significant cytokine production (Welzl *et al*., 2001; Kules *et al*., 2014). Based on severity of anaemia, uncomplicated babesiosis can be classified as mild, moderate or severe disease. Circulatory disorders are the most probable cause of mortality in canine babesiosis (Zygner *et al*., 2021). Mild uncomplicated babesiosis can progress to severe uncomplicated form, in which anaemia can become life-threatening (Jacobson and Clark, 1994). The clinical presentation of uncomplicated babesiosis includes pale mucous membranes, pyrexia, loss of appetite, lethargy, enlargement of the spleen, and a water-hammer pulse (Taboada and Merchant, 1991), whereas, clinical signs associated with complicated babesiosis are variable and related to the complications developed. The common complications associated with canine babesiosis include acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, IMHA, per acute babesiosis, acute respiratory distress syndrome (ARDS), haemoconcentration, hypotension, pancreatitis and shock (Jacobson and Clark, 1994; Lobetti, 1998). Rare complications may manifest as gastrointestinal disorders, muscle pain, eye involvement, upper respiratory symptoms, necrosed extremities, fluid retention, and chronic conditions. Various complications may occur simultaneously. Acute severe complicated babesiosis causes death in a short time (Zygner *et al*., 2021).

Anaemia: Haemolytic anaemia and thrombocytopenia are most frequent abnormalities in canine babesiosis (Muhlnickel *et al*., 2002; Boozer and Macintire, 2005; Eichenberger *et al*., 2016; Zygner *et al*., 2023). *Babesia* infect erythrocytes frequently, results in haemolytic anaemia (Taboada and Merchant, 1991). Anaemia can be severe, particularly in young and immunosuppressed dogs (Boozer and Macintire, 2005). The decrease in haemoglobin, haematocrit and total erythrocyte count has been recorded in dogs diagnosed with babesiosis (Niwetpathomwat *et al*., 2006; Selvaraj *et al*., 2010; Shah *et al*., 2011; Wadhwa *et al*., 2011; Andoni *et al*., 2013; Nalubamba *et al*., 2015; Bilwal *et al*., 2017). Significant decrease in haemoglobin, haematocrit and total erythrocyte count is indicative of severe anaemia (Gonde *et al*., 2017). However, anaemia is not always proportional to the erythrocyte lysis and level of parasitaemia (Eichenberger *et al*., 2017). Wide range of variations in haemoglobin, haematocrit and total erythrocyte count has been documented in clinical cases of *B. gibsoni* infected dogs (Varshney *et al*., 2003). Profound anaemia has been documented in low parasitaemia, which indicates that factors other than direct damage to the erythrocyte membrane by parasites are also responsible for erythrocyte lysis. Parasitaemia may result in increased osmotic fragility of RBCs (Makinde and Bobade, 1994) as well as an increase in serum haemolytic factors (Onishi and Suzuki, 1994) causing haemolytic anaemia (Jacobson and Clark, 1994). Furthermore, peripheral sludging of capillaries, compromised haemoglobin activity, oxidative injury, reduced life span of erythrocytes, splenic removal of damaged and parasitized erythrocytes, complement-mediated erythrocyte destruction, non-immune mediated destruction of erythrocytes and autoimmune erythrolysis causes anaemia (Taboada and Merchant, 1991; Jacobson and Clark, 1994; Morita *et al*., 1996; Murase *et al*., 1996; Wozniak *et al*., 1997; Taboada, 1998; Otsuka *et al*., 2002; Bilwal *et al*., 2017; Crnogaj *et al*., 2017). In babesiosis, oxidative stress can lead to damage in erythrocytes, making them more vulnerable to phagocytosis (Murase *et al*., 1996; Tvedten, 2004).

In canine babesiosis, anaemia is usually normochromic to hypochromic (Schetters *et al*., 1998; Taboada, 1998). The haemogram typically reveals macrocytic, hypochromic and regenerative anaemia (Boozer and Macintire, 2005). In *Babesia* infected dogs, initially normocytic normochromic anaemia, then macrocytic, hypochromic and regenerative anaemia was found as the disease progresses (Furlanello *et al*., 2005; Nalubamba *et al*., 2015).

Sundar *et al*. (2004) observed anaemia and mild leucopenia in dogs infected with *B. gibsoni*, along with findings of hypochromasia, polychromasia, anisocytosis and normoblasts in all the positive blood

smears, which suggest regenerative anaemia. Anisocytosis and nucleated erythrocytes in blood smears is indicative of regenerative anaemia (Gonde *et al*., 2017). The degree of reticulocytosis is directly related to the intensity of anaemia (Furlanello *et al*., 2005).

Thrombocytopenia: Thrombocytopenia is a hallmark in canine babesiosis (Kirtz *et al*., 2012; Karasova *et al*., 2022). Moderate to severe thrombocytopenia is common and most consistently reported haemostatic abnormality in canine babesiosis, regardless of the *Babesia* spp. involved (Lobetti, 2003; Taboada and Lobetti, 2006; Vannier *et al*., 2015; Solano-Gallego *et al*., 2016). Significant thrombocytopenia is observed in dogs infected with *B. gibsoni* (Salem and Farag, 2014; Gonde *et al*., 2017) and *B. canis* (Salem and Farag, 2014; Ruiz de Gopegui *et al*., 2007; Thongsahuan *et al*., 2020). This could be attributed to the sequestration of platelets in the spleen or to immune-mediated destruction of platelets, and DIC (Boozer and Macintire, 2003).

Thrombocytopenia may be transient or persistent and can occur in conjunction with other haematologic abnormalities or as a singular entity (Taboada and Lobetti, 2006). Significant thrombocytopenia and anaemia have been observed in canine babesiosis (Muhlnickel *et al*., 2002; Ruiz de Gopegui *et al*., 2007; Shah *et al*., 2011; Nalubamba *et al*., 2015, Yogeshpriya *et al*., 2018). Furlanello *et al*. (2005) observed thrombocytopenia along with leucopenia in *B. canis* infected dogs. Bilwal *et al*. (2017) found statistically non-significant differences in platelet and total leucocyte count along with anaemia in *B. gibsoni* infected dogs.

Leucopenia: The picture of leucogram is variable in canine babesiosis and less reliable in terms of characterizing the *Babesia* parasite reaction (Shah *et al*., 2011; Eichenberger *et al*., 2016). Regenerative anaemia and mild thrombocytopenia, along with neutrophilic leucocytosis, are recorded in dogs with *B. gibsoni* infection (Suarez *et al*., 2001). Variable count of leucocytes has been noted in dogs affected with babesiosis (Ruiz de Gopegui *et al*., 2007; Bartnicki *et al*., 2017). Niwetpathomwat *et al*. (2006) observed hypocytic hypochromic anaemia and thrombocytopenia with non-specific leucocyte abnormalities in canine babesiosis. Decreased values of haemoglobin, haematocrit, total erythrocyte count and neutrophilic leucocytosis with lymphocytopenia were noted in canine babesiosis (Selvaraj *et al*., 2010). Significant reduction in haemoglobin, haematocrit, total erythrocyte count and platelet count along with significant leucopenia with monocytosis was noted in *B. canis* infected dogs (Salem and Farag, 2014). In spite of variable leucocyte count, leucopenia was noted in dogs affected by babesiosis. One possible mechanism of leucopenia is extravasation of leucocytes initiated by cellular interactions between circulating leucocytes and endothelium (Butcher, 1991; Springer, 1994). The subsequent neutrophil-endothelial interaction could also trigger vascular inflammation (Mine *et al*., 2001; Baric Rafaj *et al*., 2013).

Complications and multiple organ dysfunction syndrome (MODS)

The complications and MODS associated with canine babesiosis encompass clinical signs that are not directly linked to the destruction of erythrocytes by the parasite. Severe form of complicated babesiosis occurs mainly as a result of overproduction of pro-inflammatory cytokines and is associated with high mortality (Zygner *et al*., 2014; 2015; Goddard *et al*., 2016; Leisewitz *et al*., 2019). Severe anaemia occurs in babesiosis, which results in cellular hypoxia. Reduced oxygen availability activates the expression of nuclear factor-kappa B, which is responsible for the release of pro-inflammatory cytokines (Szade *et al*., 2015). Most of the clinical manifestations associated with canine babesiosis arise from haemolytic anaemia due to the destruction of erythrocytes, accompanied by a systemic inflammatory response that may lead to dysfunction of multiple organs. Although severe anaemia and resultant hypoxia contribute to complications in babesiosis, it is presumed that systemic inflammatory mediators (generated by host tissues and inflammatory cells) are largely responsible for end-organ damage (Jacobson and Clark, 1994; Baneth, 2018). Mediators of MOD i.e., cytokines, nitric oxide and reactive oxygen species are generated by the host tissues in response to parasite rather than directly from the parasite itself (Kules *et al*., 2014). SIRS is common in cases of canine babesiosis in Africa, which is caused by the parasite *B. canis rossi* (Lobetti, 2000). Welzl *et al*. (2001) studied the implications of SIRS and MODS in dogs diagnosed with complicated babesiosis, assessing their role in determining the disease outcome. Eighty-seven percent of cases were SIRS-positive, 52% of the cases had single-organ damage, whereas 48% of cases had multiple-organ dysfunction. Dogs developing severe inflammation had a greater incidence of death than those with severe anaemia, indicating that the intensity of the

inflammatory response is the dominant mechanism in the outcome of the disease (Reyers *et al*., 1998).

Complications occur in canine babesiosis include acute kidney injury, cerebral babesiosis, coagulation disorders, jaundice and liver dysfunction, IMHA, peracute babesiosis, ARDS, relative haemoconcentration, pancreatitis, rhabdomyolysis, hypotension, myocardial pathology and shock (Mohr *et al*., 2000; Welzl *et al*., 2001; Jacobson, 2006; Schoeman, 2009).

Immune-mediated haemolytic anaemia (IMHA): *Babesia* parasite replicate inside the erythrocytes and initiates a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes (Zygner *et al*., 2007). The most significant indicator of IMHA is the persistence of haemolysis, despite the administration of suitable anti-babesial treatment. In *B. gibsoni* infection, parasitaemia is usually mild, but severe anaemia frequently develops (Onishi *et al*., 1990; Boozer and Macintire, 2005). In complicated babesiosis, autoimmune haemolytic anaemia (AIHA) can occur (Reyers *et al*., 1998). It is clinically more important than parasite-induced erythrocyte destruction, as the intensity of AIHA is contingent upon the response of the host. The exact mechanism by which *Babesia* spp. induce IMHA is unknown. The most commonly accepted theory of antibody or complement mediated haemolysis is binding of Soluble Parasite Antigens (SPA) to the erythrocyte's surfaces (Homer *et al*., 2000). It is considered to be secondary to the inappropriate antibodies production against erythrocyte membrane. Studies suggest that elevated anti-erythrocyte membrane antibodies (IgG and IgM) and erythrocyte-bound IgG are induced by *B. gibsoni* infection in dogs (Adachi *et al*., 1992;1994). The antibody levels are inversely correlated with the severity of anaemia (Adachi *et al*., 1992). The erythrophagocytic activity of macrophages increases with *B. gibsoni* infection (Murase and Maede,1990).

Diagnosis of IMHA requires demonstration of saline agglutination of RBCs or spherocytes, or both (Taboada and Lobetti, 2006). However, the lack of spherocytes and/or autoagglutination does not rule out an immune-mediated process, as these findings are observed in only 75% and 87% of cases of IMHA, respectively (Burgess *et al*., 2000). Furthermore, spherocytes and autoagglutination were observed approximately 3 to 4 weeks post-infection (Adachi *et al*., 1992). A positive Coombs test is not considered a reliable tool for the diagnosis of IMHA in babesiosis, as 84% of canine patients infected with *B. canis* or *B. gibsoni* have positive Coombs test (Farwell *et al*., 1982). Flow cytometry should be employed for the identification of anti-erythrocyte antibodies (Furlanello *et al*., 2005).

Red biliary syndrome: In certain cases of babesiosis, some dogs may exhibit a high haematocrit level even in the presence of haemolysis (relative haemoconcentration). It signifies a paradoxical complication, commonly called red biliary syndrome (Petra *et al*., 2018). In babesiosis, haemoconcentration occurs as a result of plasma loss caused through an excessively permeable endothelium (Ince *et al*., 2016). The cause is thought to be vasculitis caused by systemic inflammation and the movement of fluid from the vascular system to the extravascular space. Plasma protein concentration remains normal in red biliary syndrome (Jacobson and Clark, 1994). Haemoconcentration occurs with other complications, such as cerebral babesiosis, and an elevated risk of acute renal failure (ARF), leading to higher mortality (Botha, 1964; Welzl *et al*., 2001; Jacobson, 2006).

Antioxidant dysfunction: Oxidative stress can cause lipid peroxidation of cellular membrane phospholipids, resulting in direct injury to the cell membrane. RBC membrane is rich in polyunsaturated fatty acids (PUFA) which is highly prone to oxidative damage (Omar *et al*., 2015). Presence of parasite within erythrocyte disturbs the key antioxidant system of red cells, which leads to release of oxidant mediators from red cells and ultimately causing cell injury and haemolysis (Martusevich and Karuzin, 2015). Oxidative damage induced haemolysis results in methemoglobinaemia. Methemoglobinuria as well as methemoglobinaemia have been documented in *Babesia* infected dogs (Morita *et al*., 1996; Otsuka *et al*., 2002).

Increased macrophage production of superoxide and other reactive oxygen species (ROS) has been documented in *B. gibsoni* infection (Otsuka *et al*., 2001; 2002). Studies of experimentally induced *B. gibsoni* infections indicate that oxidative stress, driven by free radicals, is essential for the production of antibodies against erythrocyte membranes (Adachi *et al*., 1994; Morita *et al*., 1995). Furthermore, erythrocyte oxidation may enhance susceptibility to macrophage-mediated bone marrow phagocytosis (Murase *et al*., 1996; Otsuka *et al*., 2002). Lipid peroxidation of erythrocytes by ROS results in anaemia in babesiosis (Nazifi *et al*., 2011). Levels of superoxide dismutase, catalase and glutathione peroxidase are lower in dogs infected with *Babesia* (Omar *et al*., 2015), as these are antioxidant

enzymes which counteract ROS damage.

Lipid peroxidation of erythrocytes also decreases RBC membrane pliability resulting in slowed passage and further damage to the erythrocyte as it traverses capillary beds. The capillary sludging of erythrocytes, in combination with soluble parasite proteases, activates the kallikrein system, leading to the production of fibrinogen like protein (Taboada and Lobetti, 2006). This protein induces RBC aggregation and promotes vascular stasis, which leads to ischemia, thrombosis and end-organ damage. The CNS, kidney and muscle appear to be the organs most affected by the resultant tissue hypoxia (Uilenberg *et al*., 1989; Jacobson and Lobetti, 1996).

Coagulopathic dysfunction: Babesiosis causes obvious coagulopathy (Goddard *et al*., 2013). Activated platelets, haemolysis and acute phase response may contribute to the coagulation system activation. A consequence of the systemic activation of the coagulation system might be disseminated intravascular coagulation (DIC). The diagnosis of this intricate thrombohaemorrhagic condition was established through the observation of elevated thrombin-antithrombin complexes, reduced antithrombin activity, thrombocytopenia, and a shortened activated partial thromboplastin time (aPTT) in dogs with *B. canis* infection. A transient coagulopathy exhibiting abnormalities in prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimer, and thromboelastography was also found in dogs with uncomplicated *B. rossi* infections (Liebenberg *et al*., 2013). Fibrinolysis was also affected in complicated *B. canis* infections, where dogs showed a decreased concentration of fibrinolysis inhibitors, plasminogen activator inhibitor-1 and thrombin-activatable fibrinolysis inhibitor antigen which led to increased fibrinolytic activity (Kules *et al*., 2017). In addition to influencing haemostatic activity, the proinflammatory state in babesiosis also exerts an influence on the function of endothelium. Markers of endothelial activation were increased in babesiosis as a reflection of the host inflammatory response and shifted the haemostatic activity towards the procoagulant state (Baric Rafaj *et al*., 2013).

The majority of information in regard to secondary haemostasis in canine babesiosis is from *B. canis rossi*, endemic in South Africa. The occurrence of haemostatic disorders, particularly DIC, is frequently reported in South Africa (Lobetti, 2003). On the contrary, less and sometimes contradictory information is available on secondary haemostasis in babesiosis caused by *B. canis canis* or *B. canis vogeli* in Europe. A high increase of fibrinogen as well as a mild increase of aPTT has been detected in *B. canis canis* infection (Bourdeau and Guelfi, 1995; Schetters *et al*., 1997).

Hepatopathy and icterus: Liver damage is common in babesiosis affected dogs but did not significantly affect the outcome (Welzl *et al*., 2001). Hepatopathy occurs commonly in *B. rossi* infected dogs (Jacobson, 2006). Anaemia occurs commonly in cases of babesiosis which results in hypoxia causing hypoxic liver injury. Centrilobular hepatitis is the most common and severe histopathologic changes noticed in canine babesial infection due to hypoxic liver damage (Wozniak *et al*., 1997). A transient form of hepatopathy is caused by hypoxic insults, which further causes diffuse hepatocellular swelling, and has been documented in canine babesiosis (Reyers *et al*., 1998). However, Zygner and Wedrychowicz (2009) did not find any correlations between anaemia and increased aminotransferase and alkaline phosphatase (ALP) levels in *B. canis* infection.

Common hepato-biochemical profile abnormalities are hypoproteinemia, hypoalbuminemia, increased serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, hyperbilirubinemia, and electrolyte and acid-base abnormalities (hypokalaemia, hyperchloremia and metabolic acidosis) (Pages *et al*., 1990; Bourdeau and Guelfi, 1995; Vercammen *et al*., 1997a; Lobetti, 2000; Furlanello *et al*., 2005). Significant increase in AST, ALT, ALP and globulin concentration whereas, significant decrease in total protein, albumin and A: G ratio has been observed in *Babesia* infected dogs (Welzl *et al*., 2001; Zygner *et al*., 2007; Shah *et al*., 2011; Wadhwa *et al*., 2011; Yadav *et al*., 2011; Vishnurahav *et al*., 2014; Vijayalakshmi *et al*., 2014; Bilwal *et al*., 2017; Yogeshpriya *et al*., 2018). Hyperbilirubinemia in babesiosis might occur as a consequence of intravascular and extravascular haemolysis (Irwin and Hutchinson, 1991). Although the haemolytic anaemia contributes to hyperbilirubinemia, nevertheless, it is not the sole cause. Kupffer cell hypertrophy and increased numbers of CD31 lymphocytes and macrophages have been demonstrated, suggesting that immune-mediated inflammation likely plays a contributing role (Wozniak *et al*., 1997).

Jaundice has been documented in *B. gibsoni* infected dogs (Varshney *et al*., 2008). Hepatic enzymes increase frequently in icterus. Yellowish discoloration of all visible mucous membranes and skin with

hepatomegaly and hypoechoic images in the liver on ultrasonography has been documented in *B. gibsoni* infected dog (Yadav *et al*., 2011). Elevated levels of AST and ALT may result from the release of these enzymes from damaged liver parenchymal cells, which can occur due to necrosis or changes in membrane permeability, signaling hepatic dysfunction (Gupta *et al*., 2002). Significantly increased levels of ALP in cases of babesiosis may result from damaged or abnormal function of biliary system (Crnogaj *et al*., 2010).

Cardiac dysfunction: Although cardiac involvement is a rare complication in canine babesiosis (Jacobson and Clark, 1994; Lobetti, 1998), however in fatal cases, cardiac and vascular disorders may play a significant role in how the illness progresses (Bartnicki *et al*., 2017). Heart lesions have been reported as incidental findings at post-mortem examination of complicated babesiosis (Moore and Williams, 1979). Anaemia is very common in canine babesiosis and possibly plays a role in the promotion of myocardial cell injury, such as myocarditis and the subsequent development of arrhythmias (Lobetti *et al*., 2002; Lobetti, 2005; Diniz *et al*., 2008). Due to anaemia, metabolic demand on heart increases which may exceed its capacity of supplying oxygen that leads to hypoxia and myocardial infarction (Portman *et al*., 1995; Szachniewicz *et al*., 2003). A negative correlation between haemoglobin and serum troponin I level may imply that dogs suffering from severe to very severe anaemia is at a higher risk of myocardial injury (Champion *et al*., 2013). In canine babesiosis, an increase in N-terminal pro-brain natriuretic peptide (NT-proBNP) is indicative of reduced cardiac function, which becomes more severe with the advancement of the disease (Lobetti *et al*., 2012). Subepicardial and subendocardial haemorrhages, micro-thrombi of myocardium, hydropericardium and haemopericardium have been observed (Moore and Williams, 1979; Ristic and Kreier, 1984; Taboada, 1998).

The electrocardiogram (ECG) is an important diagnostic tool for the diagnosis of cardiac disorders associated to myocardial hypoxia (Tilley, 1992). Myocarditis, ventricular premature complexes (VPCs) and paroxysmal ventricular tachycardia were reported in dogs suffering from myocarditis due to *Babesia* infection (Lobetti *et al*., 2002). Changes in ECG are attributed to consequences of hypoxia, which subsequently lead to damage to the cardiac muscle (Lobetti, 2010). However, Bartnicki *et al*. (2017) stated that there is no correlation between haematological parameters and changes in ECG in canine babesiosis. It is speculated that cardiac changes in canine babesiosis may develop owing to overwhelming inflammatory response and anaemic-hypoxic state (Reyers *et al*., 1998). In canine babesiosis, either single or multiple abnormalities in the ECG have been documented (Chaudhuri *et al*., 2017). The most common ECG changes in canine babesiosis are increased heart rate, increased sinus rhythm, axis deviations, sinoatrial block, atrioventricular block, ventricular tachycardia and additional ventricular beats, low R-amplitude, prominent Q, ventricular premature complexes, prolonged QRS, ST depression, prolonged ST interval, ST coving, notching of the R wave and increase in T wave amplitude (Lobetti *et al*., 2002; Dvir *et al*., 2004; Bartnicki *et al*., 2017; Chaudhuri *et al*., 2017; Sindhu *et al*., 2020). ST deviation (depression and elevation) and ST coving are non-specific signs of myocardial ischaemia in dogs (Tilley, 1992). The occurrence of bradycardia is indicative of poor prognosis in canine babesiosis (Dvir, 2001).

Along with ECG, biomarkers such as cardiac troponin T (cTnT), cardiac troponin I (cTnI) and Creatine Kinase-Myocardial Band (CK-MB) should be estimated to assess degree of cardiac injury. Cardiac biomarkers were used to assess the degree of myocardial injury caused by non-cardiac origin like anaemia (Lobetti *et al*., 2002; Lobetti, 2005). Several investigations have indicated increased serum cTnI concentrations in anaemia (Lobetti *et al*., 2002; Lee *et al*., 2004; Ralli *et al*., 2005; Diniz *et al*., 2008; Aslam *et al*., 2009). Serum cTnI concentration is correlated with severity of anaemia (Diniz *et al*., 2008; Fartashvand *et al*., 2013). Analysis of plasma cTnI is a feasible and sensitive test and is superior to cTnT in diagnosing cardiac complications in dogs diagnosed with babesiosis (Lobetti *et al*., 2002). Significantly increased level of CK-MB was observed in dogs with dilated cardiomyopathy, mitral valve disease, idiopathic pericardial effusion, tricuspid valve disease and hypertrophic cardiomyopathy (Sagar *et al*., 2021). Increased serum activity of CK-MB is indicative of an extensive myocardial injury due to anaemia in canine babesiosis (Ishikawa *et al*., 1997; Lobetti *et al*., 2002; Lobetti, 2005; Diniz *et al*., 2007; 2008). Bartnicki *et al*. (2017) observed increased levels of CK-MB in all dogs diagnosed with babesiosis, whereas serum troponin I level was elevated in some dogs only. The tissue specificity of CK-MB has been shown not to be absolute due to its presence in skeletal muscle besides

myocardial muscle and following trauma, both types are released into the bloodstream (Apple, 1999).

Acute renal failure (ARF): ARF is a rare but life-threatening complication of babesiosis (Leisewitz *et al*., 2019). The usual warning sign of ARF in babesiosis is the presence of anuria or oliguria, regardless of adequate rehydration (Slappendel, 1979). Renal involvement is similar to the renal dysfunction observed in cases of sepsis (Jacobson, 2006). Renal involvement associated with a five times higher risk of mortality compared to all other complications in canine babesiosis (Welzl *et al*., 2001). Proteinuria and the presence of casts and renal tubular epithelial cells in the urine in canine babesiosis are suggestive of renal damage (Moore and Williams, 1979; Lobetti and Jacobson, 2001). However, it does not predict renal failure. Intravascular haemolysis may cause ARF in canine babesiosis (Pages, 1992), which causes haemoglobinuria and eventually results in haemoglobinuric nephropathy. Presence of haemoglobin in the tubule cells and renal tubules has been seen in dogs died naturally with *B. canis* infection (Zygner *et al*., 2021). A study by Zygner *et al*. (2007) suggested that renal congestion may contribute to the onset of renal hypoxia and azotaemia in dogs affected by babesiosis.

Microscopically, renal tubular epithelial cells (RTECs) can be swollen and contain droplets of haemoglobin as well as small vacuoles (Maegraith *et al*., 1957). The presence of proteinuria, renal tubular epithelial celluria, variable enzymuria, and azotaemia has been observed in canine babesiosis (Lobetti and Jacobson, 2001). Renal haemodynamics are much more likely to be abnormal when cardiac dysfunction is present. Therefore, renal congestion in dogs suffering from babesiosis is probably related to cardiac failure and increased central venous pressure (Zygner *et al*., 2021). Hypoxia is more probable cause of renal tubular injury than haemoglobinuria in dogs infected with babesiosis (Mathe *et al*., 2007; Ayoob *et al*., 2010). Renal failure is diagnosed based on ongoing evaluation of urine volume, urine analysis and nitrogen waste products (Slappendel, 1979). A significant increase in the level of BUN was recorded in dogs with babesiosis (Sudhakara Reddy *et al*., 2016; Bilwal *et al*., 2017). A disproportionate elevation in urea level is often seen in complicated babesiosis and may be caused by increased muscle catabolism (Jacobson and Lobetti, 1996). A significant increase in creatinine levels has been noted in cases of canine babesiosis (Andoni *et al*., 2013; Vishnurahav *et al*., 2014; Sudhakara Reddy *et al*., 2016). Elevations of urea, creatinine levels, and hypersthenuric urine have been documented in canine babesiosis (Furlanello *et al*., 2005; Yogeshpriya *et al*., 2018). Renal azotaemia and arterial hypotension were identified as being associated with one another in canine babesiosis (Zygner *et al*., 2014). Therefore, hypotension ought to impact hypoxia, renal ischaemia and development of acute renal failure (Walsh *et al*., 2013). In complicated babesiosis hypotension is more likely cause of hypoxic renal damage than anaemia and probably also associated with acute tubular necrosis (Zygner and Gojska-Zygner, 2014). An elevated serum urea alone is an unreliable indicator of renal insufficiency in babesiosis (Jacobson and Clark, 1994). A strong positive correlation between serum TNF-α and serum concentration of urea and creatinine suggested that TNF-α has an influence on the development of renal failure in canine babesiosis (Zygner *et al*., 2014). An ultrasonographic pattern of *B. gibsoni* infected dogs revealed mild to significant increase in echogenicity within the cortical and medullary areas, along with a relatively hypoechoic appearance at the corticomedullary junction and in the central medullary regions (Yogeshpriya *et al*., 2018).

Cerebral babesiosis: Cerebral babesiosis is infrequently observed in canine babesiosis; however, it is associated with a poor prognosis (Jacobson, 2006) and high mortality (Boozer and Macintire, 2003; Zygner *et al*., 2021). This condition is marked by nervous symptoms such as limb weakness, muscular pain, incoordination, loss of consciousness, muscle tremors, ataxia, aggression, seizures, paddling, anisocoria, central blindness, nystagmus, tetra-and paraparesis, stupor, coma and finally sudden death (Jacobson and Clark,1994; Schoeman, 2009). Cerebral babesiosis is primarily indicated by epileptic seizures (Zygner *et al*., 2021). The pathogenesis of cerebral babesiosis is associated with the sequestration of parasitized erythrocytes within the microvasculature of the central nervous system. This sequestration causes the release of inflammatory mediators and results in tissue hypoxia, which may result in neurological symptoms (Schetters and Eling, 1999; Zygner *et al*., 2021). Most dogs affected by cerebral babesiosis had congestion of grey matter, and histological examination indicates injuries, congestion, extravasations, oedema, haemorrhages, neuronal degeneration and necrosis (Jacobson, 2006; Daste *et al*., 2013; Leisewitz *et al*., 2014; Zygner *et al*., 2021). The likelihood of death is 57 times higher when the

central nervous system is involved compared to all other complications in babesiosis in dogs (Welzl *et al*., 2001).

Acute respiratory distress syndrome (ARDS): ARDS frequently occurs as a complication in severe babesiosis (Daste *et al*., 2013; Fanelli *et al*., 2013). It is a catastrophic complication that carries a high rate of mortality (Welzl *et al*., 2001; Leisewitz *et al*., 2019). Pulmonary inflammatory response may be potential cause of death in *Babesia* infection in dogs (Zygner *et al*., 2021). The pathophysiology of ARDS in canine babesiosis is probably related to noncardiogenic pulmonary oedema which may arise from DIC, overproduction of pro-inflammatory cytokines like IL-6 and TNF-α, as well as damage to type I pneumocytes induced by free radicals (Ruiz de Gopegui *et al*., 2007; Zygner *et al*., 2014; 2015; Goddard *et al*., 2016). Acute pancreatitis in complicated babesiosis may also trigger ARDS (Zygner *et al*., 2021). Clinical signs include tachypnoea, moist cough, serosanguineous frothy respiratory secretions and hypoxemia. Radiograph reveals either a diffuse or caudodorsal patchy alveolar infiltrate with normal cardiac silhouette and vessel size. Pulmonary capillary wedge pressure is normal. Multifocal deposition of immunoglobulin M antibodies within the walls of inflamed pulmonary arteries was noticed in *B. gibsoni* infection (Wozniak *et al*., 1997). In dogs infected with *B. canis*, pulmonary oedema and congestion are frequently observed post-mortem findings in lungs, and on histological examination, emphysema, interstitial inflammation, subpleural fibrosis, presence of thrombi, presence of siderophages, anthracosis and atelectasis have noticed (Zygner *et al*., 2021). The detection of siderophages within the lungs of *B. canis* infected dogs may validate the congestion of lungs (Zygner *et al*., 2021). Mixed metabolic and respiratory acid-base imbalances are commonly seen in canine babesiosis. Concurrent metabolic acidosis with respiratory alkalosis and hypoalbuminemic alkalosis has been documented in *B. canis rossi* infection (Leisewitz *et al*., 2001). Significantly higher concentration of lactate and pyruvate in non-survivors indicates severity of disease (Nel *et al*., 2004; Jacobson and Lobetti, 2005; Jacobson, 2006). Metabolic acidosis occurs due to hyperlactatemia, secondary to tissue hypoxia, and respiratory alkalosis occurs due to hypoxemia-induced hyperventilation (Leisewitz *et al*., 2001).

Hypotensive shock: In babesiosis, death occurs either due to respiratory failure characterized by rapid, fulminant disease, with a strong heartbeat or circulatory shock characterized by hypothermia and a weak heartbeat (Maegraith *et al*., 1957). Hypotensive shock occurs frequently in canine babesiosis (Maegraith *et al*., 1957; Jacobson, 2006). It can occur at any point in the disease process (Freeman *et al*., 1994; Jacobson *et al*., 2000; Lobetti and Jacobson, 2001). Incidence of hypotension upswings with disease severity but does not differentiate survivors from non-survivors (Jacobson *et al*., 2000). Shock in babesiosis is often related directly to the anaemic crisis (Button, 1979). The speculated possible causes of hypotension include parasite induced kallikrien activation with resultant vasodilation (relative hypovolemia), production of vasoactive proteins, reduced vascular tone with venous pooling, increased capillary permeability with movement of fluid to the interstitium, decreased intravascular volume and myocardial depression (Taboada and Lobetti, 2006). Myocardial infarction results in both vascular pooling and myocardial depression. Thus, a feasible explanation for hypotension in canine babesiosis could be myocardial pathology (Jacobson *et al*., 2000).

Hypoglycaemia: Hypoglycaemia is a relatively common complication of more pathogenic strains of *Babesia* spp. (Keller *et al*., 2004), but does not occur in all cases of canine babesiosis (Furlanello *et al*., 2005). Hypoglycaemia must be differentiated from cerebral babesiosis. *Babesia rossi* infection has significant correlation with poor prognosis and mortality (Keller *et al*., 2004; Nel *et al*., 2004; Jacobson and Lobetti, 2005). Collapsed state and young age patient has more chances of occurrence of hypoglycaemia (Keller *et al*., 2004). Hyperinsulinemia was once a speculated cause; however, it failed to demonstrate any significant alterations in insulin concentration (Keller *et al*., 2004). Possible causes of hypoglycaemia include increased glucose consumption secondary to anaerobic glycolysis, hypermetabolism and enhanced cellular uptake of glucose, depletion of hepatic glycogen store and hepatic dysfunction with impaired gluconeogenesis. Varshney and Hoque (2002) reported considerably low blood glucose level in hepatopathies in canine babesiosis. Strombeck *et al*. (1988) reported hypoglycaemia due to impaired carbohydrate metabolism in liver is a rare finding.

Pancreatitis: Acute pancreatitis frequently occurs as a complication associated with canine babesiosis (Mohr *et al*., 2000; Jacobson, 2006; Mathe *et al*., 2006). It

occurs commonly in patients with MODS and is associated with higher mortality (Zygner *et al*., 2021). Possible pathophysiology of pancreatitis in babesiosis include haemolytic anaemia with ischaemia-reperfusion which results in altered blood flow, hypoxia, altered lipid metabolism and pro-inflammatory cytokine production (Mohr *et al*., 2000).

Rhabdomyolysis: In canine babesiosis, rhabdomyolysis is a less frequent complication. It has been recorded in *B. canis rossi* infection (Jacobson and Lobetti, 1996). Four-fold increase in the serum activity of creatinine kinase (CK) has been found in *B. canis* infection (Furlanello *et al*., 2005), that might be attributed to rhabdomyolysis. The pathogenesis of rhabdomyolysis in babesiosis is unclear, however, inflammatory cytokines and nitric oxide are thought to potentially play a crucial role. It is characterized by muscular pain, tremors, pigmenturia and elevated concentration of myoglobin, CK and ALT (Welzl *et al*., 2001; Jacobson, 2006). Other complications accompanying rhabdomyolysis include cerebral babesiosis, acute kidney injury (AKI) and ARDS (Jacobson and Lobetti, 1996).

Splenomegaly: Diffuse enlargement of spleen with mild hypoechogenous pattern of parenchyma has been documented in dogs infected with *B. gibsoni* (Yogeshpriya *et al*., 2018). It may be attributed to diffuse proliferation of lymphocytes and plasma cells in the white and red pulp and non-specific pooling or sequestration of erythrocytes and platelets or due to abundance of hosting macrophages in spleen (Namita *et al*., 2000).

Diagnosis

Specific diagnosis of *Babesia* spp. is very important because prognosis and response to treatment are variable (Boozer and Macintire, 2005). Generally, babesiosis is suspected when anaemia and or thrombocytopenia is diagnosed in an ailing dog along with tick infestation or history of previous tick exposure. Specific identification of the *Babesia* spp. is utmost important for treatment. Traditionally, *Babesia* spp. can be diagnosed based on size and morphology of the parasite within the erythrocytes in stained blood smear under light microscopy. *Babesia gibsoni* forms round to oval piroplasm (1 to 2.5 µm), whereas *B. canis* forms pear-shaped piroplasm (2.5 to 5 µm), usually occurring in pairs (Meinkoth *et al*., 2002; Singh *et al*., 2014). The parasitized erythrocytes tend to sludge in the capillaries (Abdullahi *et al*., 1990); therefore, smears made from ear or nail capillary blood enhance the likelihood of detection of the parasite (Bohm *et al*., 2006; Irwin, 2009). *Babesia* parasite tends to preferentially infect reticulocytes over mature RBCs; hence, smears prepared from a concentrated and stained buffy coat ease the detection of the parasite (Mattia *et al*., 1993). *Babesia* spp. are easily detectable in blood smears if more than >0.1% of RBC are infected for a duration of approximately 3 to 4 weeks (Meinkoth *et al*., 2002). Microscopic examination of a well-prepared and properly stained blood smear is the easiest and most accessible diagnostic test for diagnosis of clinical babesiosis in dogs (Solano-Gallego *et al*., 2016), where infection is acute with moderate to high parasitaemia. However, it is not as effective in identifying chronic and sub-clinical cases of babesiosis in carrier dogs due to very low and often intermittent parasitaemia (Matsuu *et al*., 2005; Miro *et al*., 2015). The diagnosis by light microscopy of stained blood smear requires expertise because parasites are often overlooked when parasitaemia is significantly low (Kushwaha *et al*., 2018). The very low percentage of *Babesia*-infected cells present in peripheral blood may account for the reduced sensitivity of the microscopic detection method (Mghirbi and Bouattour, 2008; Buddhachat *et al*., 2012). Due to these limitations, serological and molecular methods should be employed to detect the parasite.

Serological tests like indirect fluorescent antibody tests (IFAT), and enzyme-linked immunosorbent assay (ELISA) are frequently employed in diagnosing canine *Babesia* spp. IFAT is a highly sensitive test for diagnosing *B. gibsoni* in dogs that are subclinically or chronically infected, even when there is a notably low level of parasitaemia (Kushwaha *et al*., 2018), but not able to differentiate between *Babesia* spp. in dogs due to cross-reactions between *Babesia* spp. and with other closely related apicomplexan parasites (Yamane *et al*., 1993). Traditional ELISA and dot-ELISA are more sensitive but significantly less specific compared to IFAT (Wanduragala *et al*., 1987). The development of recombinant ELISA has improved test specificity (Verdida *et al*., 2004). However, serology is not able to differentiate between *B. canis, T. annae* and *B. gibsoni* infection (Miro *et al*., 2015) and in the initial phases of infection, false-negative results may occur (Taboada and Merchant, 1991).

Diagnostic sensitivity has increased many folds by employing molecular methods like Polymerase Chain Reaction (PCR) (O'Dwyer *et al*., 2009). With PCR testing, species-specific amplification of regions of

parasite DNA provides a definitive diagnosis (Boozer and Macintire, 2005). Therefore, it is very useful tool for identifying the *Babesia* infection with low levels of parasitaemia, differentiation of *Babesia* spp., identifying subclinical infections and monitoring response to therapy (Boozer and Macintire, 2005). Conventional microscopic examination and simple PCR-based molecular methods were recognized as inadequate for the detection of *Babesia* parasites, especially in chronically infected dogs showing asymptomatic infection or when parasitaemia levels are very low (Mittal *et al*., 2019). PCR detects parasites in the later phases of infection, despite the presence of a significantly low level of parasitaemia or with no apparent parasite shown in blood smear (Kushwaha *et al*., 2018).

The PCR- restriction fragment length polymorphism (RFLP) technique is able to distinguish between different large *Babesia* species (Solano-Gallego *et al*., 2008), and Real Time PCR is able to detect *B. canis* subspecies in endemic areas (Costa *et al*., 2012). Semi-nested PCR test is capable of identifying and discriminating the DNA of *B. gibsoni* (Asian genotype), *B. canis vogeli*, *B. canis canis* and *B. canis rossi* (Birkenheuer *et al*., 2003b). It can detect levels of parasitaemia that are over 1,000 times lower than the approximate 0.001% parasitaemia detectable by light microscopy (Bose *et al*., 1995; Birkenheuer *et al*., 2003b). A high-resolution melting curve quantitative FRET-PCR can discriminate between *B. gibsoni*, *B. canis*, *B. vogeli* and *B. rossi* species (Wang *et al*., 2010). The sensitivity as well as specificity of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) in detecting parasites is good, and the cost and time required for analysis are less than that of standard molecular testing. The presence of a protein fraction of 51–52 kDa in the serum of all dogs infected with *B. canis* was detected by the MALDI-TOF technique (Adaszek *et al*., 2014). Two-dimensional electrophoresis and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) showed potential for using a proteomic approach not only for diagnosis but also for monitoring and outcome in canine babesiosis (Kules *et al*., 2014; 2016). Diagnosis of MODS should be done based on the specific biomarkers and diagnostic tools in complicated babesiosis.

Treatment

The treatment approach for canine babesiosis varies with *Babesia* spp. involved, severity of infection and associated complications. Diminazene aceturate, imidocarb dipropionate and phenamidine isethionate are effective treatment for large *Babesia* spp. in dogs. Small *Babesia* spp. such as *B. gibsoni* and *Babesia microti*-like spp., which are more difficult to treat, and there is no treatment of choice for small *Babesia* spp. (Boozer and Macintire, 2005; Solano-Gallego *et al*., 2016). *Babesia gibsoni* infection often exhibits resistance to treatment with imidocarb dipropionate and diminazene aceturate (Solano-Gallego and Baneth, 2011). Although several treatment protocols are employed for small forms of *Babesia* that exhibit babesiacidal properties include diminazene aceturate and imidocarb diproprionate, along with various antibiotics (doxycycline, clindamycin, metronidazole, azithromycin) and anti-malarial drug (atovaquone), but treatment is not so efficient and clinical relapses may occur frequently. Recrudescence and poor response to therapy may occur more frequently in cases involving *B. conardae* than in those involving *B. gibsoni* (Meinkoth *et al*., 2002).

For the treatment of large *Babesia* spp. such as *B. canis*, *B. rossi*, *B. vogeli*, a single dose of diminazene aceturate @ 3.5 mg/kg subcutaneously (SC) or intramuscularly (IM) or @ 3-5 mg/kg IM has proven to be the most effective approach (Penzhorn *et al*., 1995; Schoeman, 2009; Plumb, 2015). *Babesia canis* was successfully treated with diminazene aceturate @ 3.5-7 mg/kg along with supportive therapy (Taboada, 1998; Sudhakara Reddy *et al*., 2016; Sivajothi and Reddy, 2017). Diminazene aceturate is generally used for treating *B. gibsoni* although its efficacy for treating *B. gibsoni* is doubtful (Suzuki *et al*., 2007). High doses of diminazene aceturate @ 7.5-10 mg/kg are recommended for treating *B. gibsoni* infections; however, it is not permitted to be used in many countries (Taboada, 1998). Yadav *et al*. (2011) treated a dog infected with *B. gibsoni* with diminazene aceturate @ 30 mg/kg body weight. Diminazene is highly toxic (Penzhorn *et al*., 1995; Plumb, 2015) and severe side effects have been documented following its use (Sakuma *et al*., 2009). The most common side effect is the sensation of pain at the site of injection, and serious complications include ataxia, seizures and death (Plumb, 2015). Central nervous system toxicity may be a consequence of repeated administration or related to dose as it has protracted elimination (Miller *et al*., 2005). Therefore, currently, its use is limited to those clinical cases only, which are refractory to other treatments (Miller *et al*., 2005).

Phenamidine isethionate is also effective against *B. canis* @ 5-20 mg/kg administered SC once daily for 2 consecutive days (Taboada and Lobetti, 2006). It

has adverse effects including pain at the site of injection, hypotension, tachycardia and vomiting (Jacobson and Lobetti, 1996; Taboada and Lobetti, 2006). The use of imidocarb dipropionate has proven to be an effective treatment for *B. canis* @ 6.6 mg/kg IM or SC with 2 doses given two weeks apart (Plumb, 2015). Administration of a single dose of imidocarb dipropionate @ 7.5 mg/kg IM or @ 5-6.6 mg/kg or @ 7 mg/kg, IM on days 1 and 14 shows clinical improvement (Uilenberg *et al*., 1981; Penzhorn *et al*., 1995; Schoeman, 2009; Adaszek *et al*., 2015). Treatment of uncomplicated cases of *B. canis* infection with imidocarb dipropionate @ 6.6 mg/kg results in significant improvement and alleviation of clinical symptoms typically occurs within the first 24-48 hours, with a usual prophylactic protective post-therapy effect up to 4 weeks, but in some cases up to 6 weeks (Vercammen *et al*., 1997b). Complete elimination of *B. gibsoni* may not be possible by imidocarb treatment, and frequently relapse occurs (Taboada and Lobetti, 2006). *Babesia gibsoni* infected dog treated with 2 doses of Inj. imidocarb dipropionate at 5 mg/kg SC at 14 days apart showed clinical improvement but after 2 months of treatment, relapse of the disease occurred with fever, lethargy and anorexia, leading to death (Suarez *et al*., 2001). Administration of imidocarb dipropionate results in adverse effects like pain at the site of injection and manifestations of cholinergic activity such as salivation, lacrimation, vomiting, diarrhoea, muscle tremors, tachycardia and dyspnoea (Ayoob *et al*., 2010; Baneth, 2018). The rapid onset of transient salivation and diarrhoea after the intravenous administration of imidocarb can be ascribed to the activity of acetylcholine, which results in excessive salivation and an increase in the peristaltic movements of the gastrointestinal tract (Roberson, 1977). Premedication with atropine (0.05 mg/kg) SC 20 to 30 minutes before imidocarb injection is recommended (Baneth, 2018).

Antibiotics are not the treatment of choice for babesiosis. Doxycycline may provide satisfactory prophylactic efficacy against *B. canis* in dogs (Vercammen *et al*., 1996). Vishnurahav *et al*. (2017) successfully treated six dogs detected positive for *B. gibsoni* with Inj. clindamycin @ 11 mg/kg intravenously q24hr for 10 days supported with haematinics. However, a single antibiotic is not able to remove the infection. Several combinations of antibiotics were attempted by many researchers for the treatment of *B. gibsoni* in dogs. A combination of clindamycin-metronidazole-doxycycline (Suzuki *et al*., 2007) or a combination of doxycycline-enrofloxacin-metronidazole orally with and without IM injection of diminazene aceturate (Gonde *et al*., 2016) have been tried for treatment of *B. gibsoni*. The combination therapy has some efficacy against the parasite but are not effective in eliminating parasitemia. Most recent treatment regimen is atovaquone @ 13.3 mg/kg, TID administered orally in conjunction with a high-fat meal and azithromycin @ 10 mg/kg, daily orally for 10 days reported to be effective for the treatment of infections caused by *B. conradae, B. gibsoni*, and *B. vulpes* (Di Cicco *et al*., 2012; Checa *et al*., 2017). A major disadvantage of the atovaquone-azithromycin regimen is its high cost. Resistance to atovaquone associated with non-responsiveness to treatment was reported in dogs infected with *B. gibsoni* from Japan and Taiwan exhibit mutations in the cytochrome b gene of the parasite, which seemingly influence the site of action for atovaquone (Matsuu *et al*., 2006, Sakuma *et al*., 2009; Iguchi *et al*., 2012; Liu *et al*., 2016).

In many cases, medical management of babesiosis requires supportive treatment along with anti-babesial treatment. The supportive treatment includes correction of dehydration and electrolyte disturbances, and anaemia (Ayoob *et al*., 2010). Other supportive therapies may be needed depending on the organ involvement. Supportive treatment is especially important in complicated forms of babesiosis where aggressive therapy such as fluid therapy for restoring blood volume and facilitating adequate perfusion to the end organs, correction of acid-base and electrolyte abnormalities, and diuresis, blood transfusion, immunosuppressive medications for dogs diagnosed with IMHA or thrombocytopenia and heparin for DIC is required (Vial and Gorenflot, 2006; Ayoob *et al*., 2010). The use of glucocorticoids is controversial. It may reduce immune-mediated destruction, but long-term use may reduce splenic clearance of parasites and encourage ongoing infection (Taboada, 1998).

Prevention

There are several strategies, such as the use of acaricidal products targeted to transmitting ticks, chemoprophylaxis targeted against the parasites, vaccination of dogs and restriction of dogs to roaming in areas when the ticks are active, can be employed for the prevention of canine babesiosis. Frequent blood smear evaluation, serology and PCR testing of donor dog's blood for *Babesia* spp. is recommended. Blood of donor dog should be labelled negative for *Babesia* spp. after confirming by molecular assays. Tick control by the use of acaricides is most effective. More attention

is needed in season when tick activity increases. It is important to remove the attached ticks as soon as noticed to prevent the infection. Frequent visual inspection of the skin and haircoat is an effective method of tick control as the parasite can only infect a dog after more than 24 hours of attachment, provides a window of time to eliminate the tick and prevent transmission (Dantas-Torres and Otranto, 2016). Visual inspection should be combined with topical acaricide therapy to prevent tick infestation. Topical therapies of proven benefit include amitraz-impregnated collars, fipronil and imidacloprid-permethrin applied once monthly, all resulting in prevention of tick attachment or tick death within 24-48 hours (Hunter, 1997; Estrada-Pena and Ascher, 1999; Elfassy *et al*., 2001; Cruthers *et al*., 2003; Epe *et al*., 2003; Dryden and Payne, 2004; Dryden *et al*., 2006; Last *et al*., 2007). Due to more and more use of acaricides, acaricide resistance is emerging. Therefore, the development of new strategies, such as chemoprophylaxis and anti-tick vaccines, is in progress. The use of imidocarb dipropionate or doxycycline for prophylaxis against *B. canis* infection is restricted to immunosuppressed dogs in which splenectomy is done and are at risk of exposure to ticks in endemic areas (Solano-Gallego *et al*., 2016). A vaccine against *B. canis*, which incorporates exoantigens obtained from the supernatant of *in vitro* culture had developed and was available in Europe (Schetters and Montegro-James, 1995). Although the vaccine is demonstrated to be effective in reducing mortality, it is not capable of preventing infection (Schetters *et al*., 1994). A bivalent vaccine (Nobivac Piro®) containing a combination of SPA of supernatants from *in vitro* cultures of European *B. canis* isolate and South African *B. rossi* isolate, was developed, which can provide protection against both heterologous *B. canis* and *B. rossi* infection (Schetters *et al*., 2001; 2009). This bivalent vaccine received approval for utilization within the European market for a few years, but currently is not available for use. A novel protective antigen as a new vaccine candidate was discovered and produced as a recombinant protein called Canine Babesia Antigen (CBA). The CBA vaccine has a potential to replace SPA based vaccines in the future (Moubri *et al*., 2018). Effort to produce vaccines against *B. gibsoni* using 38-kDa surface protein and culture derived exoantigens is underway, but no commercial vaccine is available yet (Zhou *et al*., 2006; Sunaga *et al*., 2014).

Conclusions

Canine babesiosis has been documented amongst the most important vector-borne disease of dogs globally a long time back. Sufficient documentation regarding detection of parasites by advanced molecular techniques, along with their clinicopathology has been made. There remains a paucity of ideal treatment. There is no vaccine approved for commercial use to prevent diseases caused by *Babesia* spp. The chemoprophylactic approach results in adverse effects like anaphylaxis and kidney or liver damage; therefore, it is not recommended. In the near future, higher incidence of babesiosis can be expected due to global warming. Therefore, it is still necessary to find the pathogenesis of complications and MODS in canine babesiosis, with the objective of developing improved novel treatment and prevention strategies.

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Author's contributions: NK: Collected the required research papers and review articles for the manuscript and prepared the draft of the manuscript as per the guidelines of the journal; AM: Did the needful corrections and final editing of the manuscript.

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