Review Article

REVIEW ON PROGNOSTIC BIOMARKERS OF CANINE BABESIOSIS

Narayani Yadav[1], Raguvaran Raja[2], Debabrata Mondal [*3] and Neelam Kushwaha[4]

Division of Medicine
ICAR-Indian Veterinary Research Institute
Izatnagar, Bareilly – 243 122, Uttar Pradesh

INTRODUCTION

Babesia species is observed as the second most common parasite of blood, after Trypanosomes that infect mammals. They are considered as very common parasites of domestic dogs in the world where the tick vector is present (Matijatko *et al*., 2012) and they also cause significant morbidity and mortality (Collett, 2000; Jacobson, 2006)**.** *Babesia* was first reported in cattle in Rumania by Babes in 1988. There are over 100 species of *Babesia* reported now in vertebrate hosts. *Babesia canis* is the larger form and is less pathogenic than smaller form of *Babesia* i.e. *Babesia gibsoni.* Multiple organs are affected in *B. gibsoni* infections and the disease has been classified as either complicated or uncomplicated, in a similar way to falciparum malaria in humans (White *et al.,* 2014). Complications reported include anaemia (Scheepers *et al.,* 2011), haemolysis, haemoconcentration (Jacobson, 2006), icterus (Jacobson and Clark, 1994), hypoglycaemia and hyperlactatemia (Keller *et al.,* 2004; Nel *et al.,* 2004). Acute lung injury is considered as a common cause of death by private practitioners in South Africa (Collett, 2000). The cerebral form of the *B. rossi* disease is very rare. Acute kidney injury is reported in a small group of dogs with complicated disease. ECG abnormalities, myocardial haemorrhage, pericardial effusion and elevated cardiac troponins have been reported (Dvir *et al.,* 2004; Lobetti *et al.,* 2002). A consumptive coagulopathy and excessive pro-inflammatory response are also noticed (Goddard *et al.,* 2016). *Babesia gibsoni* is difficult to treat. The aim is to investigate set of parameters/ abnormalities consistently associated with severe disease which were most accurate at predicting an adverse outcome to facilitate better case classification.

Epidemiology and geographical distribution

Babesia genus belonging to Phylum Apicomplexa order Piroplasmida and family Babesiidae. Morphologically canine *Babesia*

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[1]Ph.D. Scholar, Division of Medicine, ICAR-IVRI
[2] Scientist (SS), Division of Medicine, ICAR-IVRI
[3] Scientist, Division of Medicine, ICAR-IVRI, corresponding author Email id: dbmondal@gmail.com
[4]Hospital Registrar, TVCC, College of Veterinary and Animal Sciences, Udgir, Maharashtra

are categorised into small and large forms that exhibits worldwide distribution. Large form of *Babesia* infecting dogs is *Babesia canis, Babesia vogeli* and *Babesia rossi*. In India *Babesia gibsoni* piroplasm was first time reported in dogs in 1910 (Patton, 1910). Since then, there was existence of sporadic reports on microscopic detection of *B. gibsoni* in India (Karunakaran *et al*., 2011; Tresamol *et al.,* 2013). These three species are antigenically different and are transmitted by different vectors which differ widely in geographic distribution and pathogenicity (Table 1). *Babesia vogeli* is least pathogenic and it occurs in Japan, Australia, France, South Africa, Brazil, and USA (Matjila *et al*., 2004). *Babesia canis* appears either as a single or pairs and is widespread in Europe. *Babesia rossi* occurs mainly in Southern Africa and is apparently most pathogenic subspecies. Improved PCR techniques have recently used for better differentiation of these parasites (Matjila *et al*., 2008). *Babesia gibsoni* is a small organism, measuring 1-3.2 µm and is pleomorphic, having variety of intraerythrocytic forms; oval or signet shaped. *Babesia gibsoni* occurs mainly in the Japan, North Africa, Middle East, southern Asia, South America and is an emerging parasitic disease in the USA (Muhlnickel *et al*., 2002). A more virulent subspecies of *B. gibsoni* has recently been identified in California (Kjemtrup *et al*., 2006) is *B. annae.* Recently, two additional canine *Babesia* spp have been noticed in the United States; *Babesia conradae*, a small piroplasm and an unnamed large piroplasm (Conrad *et al*., 1991; Birkenheuer *et al*., 2004; Kjemtrup *et al*., 2006).

Transmission

Different species of ticks such as *Dermacentor*, *Rhipicephalus sanguineus*, and *Haemaphysalis ellipticum* can transmit the large form of *Babesia* to dogs, whereas *Haemaphysalis bispinosa* and *Haemaphysalis longicornis* transmit *B. gibsoni*. *B. annae* is thought to be spread by *Ixodes hexagonus* (Lobetti, 2006). Both transstadial and transovarian transmission can occur and ticks are believed to remain infective for several generations. *Babesia* spp. can also be transmitted by blood transfusion. Strong circumstantial evidence exists that *B. gibsoni* is transmitted by dog bites (Birkenheuer *et al*., 2005), whilst transplacental transmission from dam to offspring has recently been proven as an additional mode of transmission (Fukumoto *et al*., 2005).

Pathogenesis of canine babesiosis

Severity of the babesiosis depends on the age, immune status of the host, species of *Babesia* and the presence of concurrent infections. Presentation of disease varies from peracute to chronic or even subclinical. *Babesia* causes disease mainly in young dogs, but dogs of all age group can be affected. The incubation period for *B. canis* ranges from 10-21 days and 14-28 days for *B. gibsoni*. After infection, different immune responses are generated, depending upon the host factors. Parasitic antigen present on the cell membrane of the infected erythrocytes leads to antibody production, opsonisation, and removal of infected cells by mononuclear phagocytic cells of haemolymphatic system (Taboada and

Table 1. Geographical distribution of *Babesia* spp in canine and their tick vectors

Disease agents	Geographical distribution	Tick vectors	References
B. canis	Worldwide	*Rhipicephalus sanguineus, Dermacentor reticulatus, Dermacentor marginatus*	Zahler *et al.,* (2000), Welzl *et al.,* (2001)
B. canis vogeli	Worldwide	*Rhipicephalus sanguineus*	Zahler *et al.,* (2000), Welzl *et al.,* (2001)
B. canis rossi	Southern Africa	*Haemaphysalis leachi*	Zahler *et al.*, (2000), Welzl *et al.,* (2001)
B. gibsoni	Africa, Asia, USA, Southern Europe, Middle East	*Haemaphysalis bispinosa, R. sanguineus*	Zahler *et al.,* (2000), Welzl *et al.,* (2001)

Lobetti, 2006). Although survivors remain chronic carriers without showing any clinical signs as the immune system is unable to completely eradicate the infection.

Canine babesiosis can be classified as uncomplicated or complicated (Ayoob *et al.,* 2010). In babesiosis, different clinical signs were described earlier including anaemia, lethargy, tachycardia, tachypnea, pale gums, red colour urine, jaundice, enlarged lymph nodes (Reddy *et al.,* 2016). The main clinicopathological findings in canine babesiosis indicate that it typically causes haemolytic anaemia, neutrophilia, lymphopenia, moderate to severe thrombocytopenia and multiple organ dysfunctions (Suresh *et al.,* 2017). The clinical signs in complicated presentation are haemolytic anaemia, cardiovascular, respiratory, hepatic, renal, gastrointestinal, neurologic and coagulopathic dysfunction. It is thought that the clinical signs may be due to anaemia, tissue hypoxia and concomitant systemic inflammatory response syndrome (SIRS) that is caused by marked cytokine release (Lobetti, 2006).

Extravascular and intravascular haemolysis take place but the progression of the anaemia is incompletely understood. Parasites directly attacks on the erythrocyte cell membrane, resulting in increased osmotic fragility which further leads to intravascular haemolysis (Makinde and Bobade, 1994). The anaemia is not related to the degree of parasitemia and dogs start to show improving clinical signs after parasiticidal treatment, even though their haematocrits generally decreased further, before starting to rise (Jacobson *et al.,* 1996). Some cases show additional immune mediated breakdown of RBC and dogs that show in-saline-positive RBC agglutination have to be carefully monitored for rapid decrease in haematocrit. It is commonly seen

in both *B. canis* subsp. *rossi* and *B. gibsoni* infection and is the most consistently reported hemostatic abnormality (Taboada and Lobetti, 2006).

Lymphopenia and thrombocytopenia could be due to sequestration of platelets in the spleen or immune mediated destruction of platelets (Shah *et al.,* 2011). In uncomplicated babesiosis, hemostatic changes denote a procoagulant state that is mostly reversed after antiparasitic therapy. In babesiosis, Antithrombin III activity is decreased due to negative acute phase response, decreased synthesis in liver, degradation by elastase from activated neutrophils and reduced availability of glycosaminoglycans. Procoagulant state and haemostatic shift towards thrombin formation are also demonstrated by elevated TAT levels (Josipa *et al.,* 2017). Two dominant pathogenic mechanisms of babesiosis are haemolysis and acute phase response. Pro-inflammatory state occurs in babesiosis that is associated with increased levels of markers of endothelial cell activation and altered hemostasis.

Organ involvement in babesiosis

Anaemia is the predominant clinical syndrome, and development of anaemia is multifactorial in canine babesiosis. Babesia directly damages the cell membrane of erythrocyte resulting in increased osmotic fragility and intravascular haemolysis (Makinde and Bobade, 1994). Immune-mediated haemolytic anaemia (IMHA) may occur secondary to the inappropriate production of anti-erythrocyte membrane antibodies, and is assumed to occur with all *Babesia* spp. Elevated concentrations of antierythrocyte membrane antibodies and erythrocyte bound immunoglobulin G have been documented in dogs infected with *B. gibsoni* (Adachi and Makimura, 1992; Adachi *et al*., 1994). Continued haemolysis, despite appropriate anti-babesial therapy, is the most prominent feature of this complication. Diagnosis requires demonstration of saline agglutination of RBCs or spherocytosis, or both (Taboada and Lobetti, 2006).

Multiple Organ Dysfunction Syndrome (MODS) can occur after infection with most pathogenic *Babesia* species, particularly *B. canis* subsp. *rossi* and *B. gibsoni* (Gomes *et al.,* 2011) Multiple organ dysfunctions in babesiosis are reported to be the end result of tissue inflammation that is initiated by several factors which include infectious organisms, hypotension and septic shock (Lobetti, 2005). Babesiosis is capable of causing severe tissue hypoxia which in turn, leads to widespread release of inflammatory mediators and tissue damage (Lobetti, 2005). The systemic inflammatory response syndrome eventually leads to multiple organ dysfunction syndrome (Welzl *et al.,* 2001). Systemic inflammatory response syndrome (SIRS) in babesiosis was characterised by increases in paraoxonase 1 and apo A-I, whereas MODS described with decrease of complement inhibitors leading to prolonged complement activation, tissue damage and also causing decrease of vitamin D binding protein due to haemolysis and activation of the coagulation

cascade (Kules *et al.,* 2016). Neutrophil/ lymphocyte ratio is a better diagnostic index to detect complications in babesiosis and also that multiple organ dysfunction (Omobowale *et al.,* 2017).

Early detection of SIRS and MODS is of major importance in clinical practice for providing information about severity and outcomes of the disease and therapy. The complications include red biliary syndrome, disseminated intravascular coagulopathy (DIC) thrombocytopenia, acute renal failure (ARF), hepatopathy, rhabdomylosis, noncardiogenic pulmonary edema, CNS dysfunction, pancreatitis, systemic hypotension, cardiac dysfunction, hypoglycaemia, hypoxemia and metabolic acidosis with hyperlactatemia (Jacobson and Clark, 1994; Jacobson and Lobetti,1996; Lobetti *et al.,* 1996; Uilenberg *et al.,* 1989; Mohr *et al.,* 2000; Leisewitz *et al.,* 2001; Welzl *et al.,* 2001; Keller *et al.,* 2004; Jacobson, 2006).

Kidney: The usual warning sign of acute renal failure (ARF) in babesiosis is anuria or oliguria despite adequate rehydration. ARF occurs in older dogs, and the prognosis is better in young dogs. Causes of renal hypoxia are anaemia, capillary sludging and systemic hypotension with compensatory renal vasoconstriction. Immune mediated membranoproliferative glomerulonephritis also leads to renal damage. Blood urea nitrogen (BUN) is an unreliable measure of renal dysfunction as the BUN measurement is being affected by intravascular haemolysis. Serum creatinine is a useful diagnostic tool of renal dysfunction as it is unaffected by haemolysis (De Scally *et al.,* 2006). Proteinuria, renal tubular casts and epithelial cells in urine sediment are commonly observed in both complicated and uncomplicated babesiosis but do not necessarily reflect renal failure (Lobetti and Jacobson, 2001).

Hepatobiliary: Icterus is reported to occur in advanced cases of canine babesiosis. Centrilobular hepatitis and severe histopathologic changes are noticed in canine babesiosis (Zakim, 1985; Wozniak *et al*., 1997). Kuffer cell hypertrophy and increased numbers of CD31 lymphocytes and macrophages have been noticed which suggested that immune mediated inflammation plays an important role (Wozniak *et al.*, 1997). Ongoing hepato-cellular damage is characterized by elevation of bilirubin, ALT and AKP levels which is due to hepatic hypoxia (Suresh *et al.,* 2017). Decline in hepatic functions coincides with decline in albumin level.

Respiratory system: Acute respiratory distress syndrome (ARDS) is a common complication of more pathogenic strains of *Babesia* species (Jacobson and Clark, 1994). The pathogenesis of ARDS includes a systemic inflammatory response syndrome, secondary to the production of inflammatory cytokines and reactive oxygen species. The clinical signs include tachypnoea, moist cough, serosanguineous frothy respiratory secretions and hypoxemia. Radiograph reveals either a diffuse or caudo-dorsal patchy alveolar infiltrate with normal cardiac silhouette and vessel size. Pulmonary capillary wedge pressure is

normal when measured. Multifocal deposition of immunoglobulin M antibodies within the walls of inflamed pulmonary arteries noticed in *B.gibsoni* infection (Wozniak *et al.*, 1997). This ARDS is a catastrophic complication and is associated with the marked elevation in the mortality (Mohr *et al*., 2000; Welzl *et al*., 2001; Jacobson, 2006).

Nervous system: The pathological changes reported most frequently in the brains of dogs with cerebral babesiosis are congestion, macroscopic and microscopic haemorrhages and sequestration of parasitised erythrocytes in capillary bed. Clinical signs resolve in some patients after antibabesial therapy and development of neurological signs is associated with high mortality rate (Mohr *et al*., 2000; Welzl *et al*., 2001; Jacobson, 2006).

Pancreas: Acute pancreatitis is a common occurrence in canine babesiosis (Mohr *et al*., 2000; Mathe *et al*., 2006; Jacobson, 2006). Pancreatitis more commonly noticed in patients suffering from MODS and was associated with an 18% increase in mortality. Clinical signs in pancreatitis are inappetance, vomition, diarrohea and abdominal pain. The pathogenesis of pancreatitis includes ischemic- reperfusion altered blood flow and oxygen delivery due to hypotensive shock, anaemia and hemoconcentration, altered lipid metabolism and pro-inflammatory cytokine production (Mohr *et al*., 2000).

Heart: In canine babesiosis, cardiac dysfunction has been recognised as a rare complication, with the majority of lesions reported as incidental findings at post-mortem examination. Macroscopic cardiac lesions include pericardial effusion and pericardial, epicardial, and endocardial haemorrhage, that mainly affects one or more chambers with the left ventricle being most commonly affected. Lesions may be multifocal but more commonly restricted to one area within the myocardium (Dvir *et al*., 2004). Cardiac dysfunction results into decreased renal blood flow and glomerular filtration due to redistribution of blood flow which is common in early stage heart failure (Lobetti and Jacobson, 2001). Cardiovascular assessment in *Babesia* infected patients should be done on the basis of echocardiography and certain cardiac biomarkers like troponin I and CK-MB. The ECG findings shows sinus tachycardia, interpolated ventricular beat and also elevated levels of troponin I and CK-MB in *Babesia* infected dogs (Bartnicki *et al.,* 2017).

Prognostic biomarkers

1) Haemato-biochemical parameters

Haemato-biochemical changes could be beneficial in determination of the severity of babesiosis in dogs. Blood films showed anisocytosis and nucleated erythrocytes indicating regenerative anaemia. Blood parameters of the affected dogs revealed significant decrease in Hb, TEC, PCV and thrombocytes. Significant decrease in lymphocytes was found in *B. gibsoni* affected animals. The affected dogs showed significant increase in serum bilirubin, ALT, AKP, BUN and creatinine (Suresh *et al.,* 2017). Non-significant increased level of haemoglobin, PCV, total erythrocyte counts and TLC

was noticed in *Babesia* infected dogs after treatment with different therapeutic protocols. Non-significant decreased level of ALT, ALP was reported in doxycycline along with clindamycin and metronidazole treated dogs (Narayani *et al.,* 2021).

Oxidant and antioxidant parameters

Overproduction of free radicles cause damage to nucleic acids, lipids, proteins and other cellular components or disproportion between scavenging and radical generating mechanism leads to oxidative stress resulting into increased lipid peroxidation (Erejuwa *et al.,* 2013; Omar *et al*., 2015)**.** RBC membrane is rich in polyunsaturated fatty acids (PUFA) which is highly prone to oxidative damage in babesiosis resulting into direct damage to red cells (Omar *et al*., 2015). 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, activate the kallikrein system leading to 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. Parasitic load is positively correlated with oxidative stress (Kalyan *et al.,* 2015). In recent years, in babesiosis, the pathogenesis of anaemia is correlated with lipid peroxidation and oxidative process (Nazifi *et al*., 2011).

Presence of hemo protozoan infection inside the RBCs disturbs the key anti-oxidant 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). Anti-oxidant elements such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) plays a vital role in counteracting ROS damage thus level of superoxide dismutase, catalase and glutathione peroxidase are lower in *Babesia* infected dogs (Kalyan *et al.,* 2015; Omar *et al*., 2015). The dogs treated with combination therapy had elevated level of catalase and SOD and decreased level of LPO on post therapy (Narayani *et al.,* 2021).

Coagulation abnormalities: *Babesia gibsoni* infection may cause disturbance in the blood coagulation mechanism which results in disseminated intravascular coagulopathy (DIC) and further leads to multiple organ failure (Rafael *et al.,* 2007). International Society on Thrombosis and Hemostasis (ISTH) established criteria for the definition of DIC in humans that includes procoagulant activation, increased fibrinolytic activity, and inhibitor consumption. Disseminated intravascular coagulation is a type of syndrome resulting from systemic generation of thrombin. Diagnosis is made by finding at least 3 of 4 abnormalities in laboratory values, namely prothrombin time, platelet count, fibrinogen and fibrinogen/fibrin degradation products (Wada *et al.,* 2003). Normal platelet count is 1.5-4 lakhs/cumm and Prothrombin time is less than 15 seconds in dogs. The mechanisms related to thrombocytopenia are not yet fully understood in babesiosis; multiple mechanisms, including platelets sequestration

in the spleen, immune-mediated destruction of platelets and development of disseminated intravascular coagulation (Subapriya *et al.,* 2020). Non-significant elevated level of platelet count was noticed post therapy in all dogs treated with different combination therapy (Narayani *et al.,* 2021).

vWF is a large multimeric glycoprotein normally found in blood plasma and produced constitutively as ultra-large vWF in endothelium (in the Weibel-Palade bodies), or in granules of megakaryocytes, and subendothelial connective tissue. *Babesia. gibsoni* parasite damages the endothelium of blood vessels which release von Willebrand factor (vWF) that leads to a consumptive thrombocytopenia (Levy *et al.,* 2005). vWF and propeptide levels were measured by using ELISA kit. Median vWF and propeptide levels were significantly greater in patients with parasitic diseases (Hollestelle *et al.,* 2006; Narayani *et al.,* 2021). Significantly decreased ($p<0.05$) PT along with decreased vWF level after triple combination therapy denoted minimal vascular endothelial cell damage and less chance for development of DIC (Narayani *et al.,* 2021).

Ultrasonography

Nyland and Hager (1985) observed normal hepatic parenchyma using USG. Normal liver appears coarser in echotexture and less echogenic than spleen and hyperechoic or isoechoic when compared to right kidney (Lamb *et al.,* 1990). In *Babesia gibsoni* there will be hepatopathy, splenomegaly and multiple organ dysfunctions. Ultrasonography can be an adjunct for diagnosis and monitoring of canine babesiosis and its systemic complications (Fraga *et al.,* 2011). The detection of diffuse heterogeneous splenomegaly can support the diagnosis of *Babesia* infection, because of the high prevalence of this lesion in these patients. The most common finding in *B. gibsoni* infected dogs was reported to be splenomegaly with a diffuse heterogenic parenchyma and generally reduced echogenicity. Diffuse hypoechoic hepatomegaly and bilaterally increased cortical echogenicity of the renal parenchyma have been reported more frequently in severe uncomplicated and complicated babesiosis (Sharma *et al*., 2001). Sharma *et al*. (2001) also reported hypoechoic enlarged liver with round caudal edge indicating hepatomegaly and diffuse enlargement of spleen with distended splenic vessels using ultrasonography in *B. gibsoni* infected dogs.

Diagnosis

Taboada and Lobetti, (2006) concluded that definitive diagnosis of *Babesia* infections require demonstration of organisms within RBCs. The smears prepared from capillary blood, such as the ear tip or nail bed enhances the likelihood of detection of organism, because the parasitized erythrocytes tend to sludge in the capillaries (Abdullahi *et al*., 1990). Similarly, examination of smears prepared from a concentrated and stained buffy coat (Percoll gradient separation) may facilitate diagnosis, as *Babesia* organisms preferentially parasitize reticulocytes over mature RBCs (Mattia *et al*., 1993). Light microscopic

examination of blood smears is an excellent diagnostic tool for acute infections with moderate or high parasitemia. Conventional microscopic examination and simple PCR based molecular detection methods for these apicomplexan parasites were found to be inadequate especially in chronically infected animals manifesting asymptomatic infection (Mitesh *et al*., 2019). Fukumoto *et al*. (2001) mentioned that PCR targets parasitic DNA rather than anti-babesial antibodies, and is therefore a reliable diagnostic tool in peracute, acute, and chronic form. PCR testing has recently become available and is very useful tool in assessing the infective species, identify low levels of parasitemia, recognizing subclinical infections, and monitoring response to therapy (Boozer and Macintire, 2005). Although, conventional/simple PCR protocols are considered to be ideal methods for laboratory diagnosis of canine babesiosis but Mitesh *et al.* (2019) stated that nested PCR instead of simple PCR be relied upon as a screening assay and also for epidemiological studies as statistically significant false-negative animals were observed among the later results. Serological tests including indirect fluorescent antibody test (IFAT) for diagnosis of babesiosis are less popular due to cross reactivity with other closely related piroplasmids (Irwin, 2009). Recently, with the advancement of the nucleic acid amplification techniques like PCR, loop mediated isothermal amplification (LAMP) assay and quantitative PCR (Mandal *et al.,* 2015; Qurollo *et al.,* 2017) diagnostic sensitivity has increased manifold in recent years.

Treatment

The primary goals are to eliminate the parasite and reverse the life-threatening anaemia. Line of treatment for canine babesiosis varies with the severity of infection and accompanying complications. Small *Babesia* spp are generally more difficult to treat (Boozer and Macintire, 2005). No single drug has been reported effective against of *B. gibsoni* in dog (Narayani *et al.,* 2021). Several drugs have been tried in the management of *B. gibsoni* infection, including babesiacidal agents (diminazene aceturate, imidocarb diproprionate), antibiotics (doxycycline, clindamycin, metronidazole, azithromycin), and an antiprotozoal agent (Atovaquone) (Bandula *et al.,* 2012; Sakuma *et al.,* 2009). Although diminazene aceturate has been used as a first-line agent for the treatment of *B. gibsoni* infection in dogs (Sakuma *et al.,* 2009), it cannot completely eliminate *B. gibsoni* from infected dogs, and relapses often occur (Hwang *et al.,* 2010; Narayani *et al.,* 2021). It is reported that relapse of canine babesiosis might be due to the resistance of diminazene aceturate in *Babesia gibsoni* parasites (Sakuma *et al.,* 2009).

Large form of babesiosis such as *Babesia canis, Babesia rossi, and Babesia vogeli* are quite sensitive to diminazine aceturate and imidocarb (Kirk *et al.,* 2017). Imidocarb dipropionate (Imizol) is another conventional therapy for canine babesiosis given at 5 - 6 mg/ kg by subcutaneous or deep intramuscular injection and two doses are given 2 weeks apart. In addition, when dogs

are simultaneously infected with a second parasite, *Ehrlichia canis*, imidocarb can affect both parasites (Pasa *et al.,* 2011). Yamasaki *et al.* (2011) have reported that nystatin; a membrane-active polyene macrolide antibiotic and an antifungal compound could destroy *B. gibsoni* by its ionophorous activity. Amphotericin B administered @ 0.5 and 1mg/kg intravenous to *B. gibsoni* infected dogs has reduced parasitemia and recurrence of parasitemia had been observed, indicating that amphotericin B did not eliminate parasites completely (Yamasaki *et al.,* 2014). Atovaquone is a novel antiprotozoal compound that has broad-spectrum activity against protozoan pathogens including *Babesia* species (Matsuu *et al.,* 2008; Sakuma *et al.,* 2009). The mechanism of action of atovaquone against other protozoa is believed to involve the inhibition of cytochrome b and electron transport (Sakuma *et al.,* 2009)**.** Treatment with azithromycin @ 10 mg/kg every 24 hours orally and atovaquone @13.3 mg/kg every 8 hrs orally for 10 days reported to be effective against *B. gibsoni* (Matsuu *et al.,* 2008; Sakuma *et al.,* 2009). Newer successful treatment modalities have been reported to have encouraging response in cure of *B. gibsoni* infection in dogs which includes, triple combined therapy with doxycycline @ 10 mg/kg along with clindamycin 25 mg/kg and metronidazole @ 5-7 mg/kg PO in combination with need based supportive therapy had better prognosis cum survivability of animal (Narayani *et al.,* 2021). This combination boosts the innate immunity and is known as the Marshall Protocol. The combination therapy might be more effective rather than a single therapy (Narayani *et al.,* 2021). However, the combination treatments are generally used as the second-line of treatment when conventional therapies fail. Meanwhile, there are many reports of development of antibiotic resistance for those antibiotics, for example, emergence of clindamycin-resistant in methicillin-resistant *Staphylococcus aureus* (Rich *et al.,* 2005), metronidazole-resistant *Trichomonas vaginalis* (Cudmore *et al.,* 2004) and doxycycline-resistant strains of *Streptococcus* (Chalker *et al.,* 2012) in animals. Thus, combination treatments of these antibiotics against babesiosis warrant further studies due to possible emergence of not only antibiotic resistant bacteria but also *Babesia.* Ultimate success of any treatment protocol lies with the positive prognostic response correlating the instituted therapy. Hence, it is much essential to evaluate the regimens of novel drug combinations for its synergistic effects if any against *B. gibsoni* infection with special emphasis on prognostic biomarkers as a success indicator of any effective therapy.

CONCLUSION

Babesiosis is a tick borne hemoprotozoan disease of canines and anaemia is the predominant clinical syndrome. This disease causes considerable morbidity and mortality in canines. Several factors such as leukocyte activation, vascular endothelial cell injury and release of chemical mediators lead to multi-organ failure and coagulation abnormality in complicated form of babesiosis. The assessment of these biomarkers is used as

indicators of severity of disease and outcome in dogs with MODS in babesiosis. Successful treatment of babesiosis is challenging task and no drugs have been demonstrated to clear *B. gibsoni* (Asian genotype) infection. Newer successful treatment has shown encouraging response in cure of *B. gibsoni* infection which includes combination of clindamycin, metronidazole and doxycycline.

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