RESEARCH ARTICLE

Haemato-Biochemical Changes in Dogs Affected with Immune Mediated Haemolytic Anaemia Caused by *Babesia gibsoni*

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Abstract

The present study was conducted in dogs (n=18) presented to small animal OPD, Department of Veterinary Medicine in Bangalore with clinical signs of canine babesiosis. Eighteen dogs were primarily screened for *Babesia gibsoni* infection by blood smear examinations and then confirmed by PCR. Further these dogs were subjected to saline agglutination test, spherocytosis in blood smear examination and Coombs test for diagnosis of immune mediated haemolytic anaemia (IMHA). The variations in haematological and serum biochemical analysis of dogs affected with IMHA caused by *Babesia gibsoni* were studied. Haematology revealed significant increase in TLC and a significant decrease in mean TEC, Hb, PCV and platelet count. Biochemical analysis revealed significant increase in mean ALT, ALP, total bilirubin, creatinine, BUN; significant decrease in mean albumin level and non-significant decrease in total protein with non-significant elevation in globulin levels.

Key words: *Babesia gibsoni*, Coombs test. Immune mediated haemolytic anaemia (IMHA), Saline agglutination test, Spherocytosis.

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Introduction

Canine babesiosis is a tick-borne, haemoparasitic disease caused by intraerythrocytic protozoa of the genus Babesia, within the phylum Apicomplexa. *Babesia gibsoni* is transmitted by the ticks *Rhipicephalus sanguineous* and *Haemophysalis longicornis*. The tick *H. longicornis* serves as a potential vector for *B. gibsoni. Babesia gibsoni* is distributed throughout the world including Middle East, Northern Africa, and South Asia. The most common species that cause canine babesiosis include *Babesia canis* and *Babesia gibsoni*, which could be differentiated based on their size within the parasitized erythrocytes. *Babesia canis* is the large form (2.5-5.0 µm), while *Babesia gibsoni* is a small pleomorphic organism (1-2.5 µm) and appears most commonly as ring form (Obeta *et al.,* 2020). *Babesia canis* is transmitted by tick *Dermacentor reticulatus*, whereas the ticks *R. sanguineous* and *H. longicornis* serve as a potential vector for *Babesia gibsoni.*

The pathogenicity of Babesia organisms is determined primarily by the species and strain involved. When red blood cells are infected, they incorporate parasite antigens on their surface-induced host-opsonizing antibodies that mark infected erythrocytes for elimination by the host mononuclear phagocyte system. In addition, the soluble parasite antigens can attach to the surface of some uninfected red blood cells and platelets. This may lead to their opsonisation by antibodies, with or

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without complement, and account for the haemolytic anaemia and thrombocytopenia, which might not always directly correspond to the level of parasitemia. Hosts can develop antibodies targeting their own erythrocytic membrane antigens, leading to erythrophagocytic activity of macrophages, contributing to immune-mediated anaemia. This study was targeted to evaluate the haemato-biochemical changes in dogs affected with immune mediated haemolytic anaemia (IMHA) caused by *Babesia gibsoni*.

Materials and Methods

Dogs presented to the Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with a history of tick infestation, high fever, pale/icteric mucous membrane inappetence, anaemia, lethargy, vomiting, diarrhoea, haemoglobinuria were screened by saline agglutination test, Spherocytosis and Coombs test for diagnosing IMHA. These dogs were screened for *B. gibsoni* infection by microscopic examination of blood smears and PCR.

Polymerase Chain Reaction (PCR)

DNA was extracted from the whole blood sample using QIA amp DNA blood mini kit (M/s QIAGEN Germany) according to manufacturer's instructions. DNA samples were amplified using *B. gibsoni* species specific Forward primer Gib599: 5'-CTCGGCTACTTGCC TTGTC-3' and Reverse primer Gib1270: 5'- CCGAAACTGAAATAACGGC-5' (Inokuma *et al.,* 2004) procured from M/s Barcode Biosciences, Bangalore. The specific amplicon size of 662 bp obtained by 1.2% agarose ethidium bromide gel electrophoresis. To amplify 18S rRNA gene of *B. gibsoni* a total 20 µL reaction mixture employed consisted of template DNA 1.5 µL, forward and reverse primers 1.5 µL each, master mix 12 µL, and nuclease free water 3.5 µL, The PCR cycle conditions used in the thermal cycler (Eppendorf, Germany) were initial denaturation at 95°C for 5 min, followed by 28 cycles of denaturation at 95°C for 1 min, Annealing 58°C for 1 min, extension at 72°C for 1 min, and Final extension at 72°C for 5 min.

Eighteen dogs infected with *B. gibsoni,* positive for saline agglutination test, Spherocytosis and Coombs test were selected for the study.

The collected blood samples were processed for laboratory assessment of the detailed haematological parameters using BC-2800 Vet, Auto-haematology analyzer. The serum samples were processed for the biochemical parameters, *viz.,* alanine amino transaminase (ALT), creatinine, BUN, alkaline phosphatase (ALP), total protein, serum albumin, globulin and total bilirubin by using semi-automatic serum biochemistry analyzer (RX-50 of Micro Lab.). The haemato-biochemical findings of affected dogs were compared with those analysed from 6 healthy dogs by unpaired 't' test.

Results and Discussion

Diagnosis of *B. gibsoni* in Dogs

A total of 18 samples were collected from dogs exhibiting clinical signs of *B. gibsoni.* The blood samples were examined for the presence of *B. gibsoni* organism in the thin blood smear stained with Giemsa stain. The microscopic examination of the blood smears showed that 14 samples out of 18 samples were positive for *B. gibsoni* organisms.

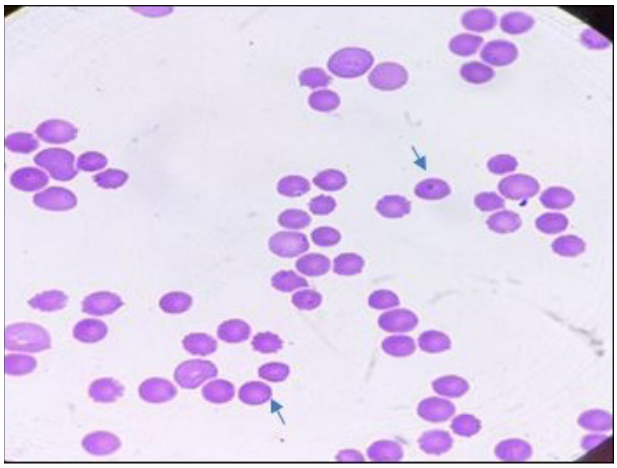

Fig. 1: Giemsa stained blood smear showing *B. gibsoni* organism

In PCR, the band amplified in line with the 662 bp band of the positive control for *B. gibsoni* organism was used to identify the organism. A UV transilluminator was used to visualise the 662 bp bands that were formed in all the positive samples for *B. gibsoni*.

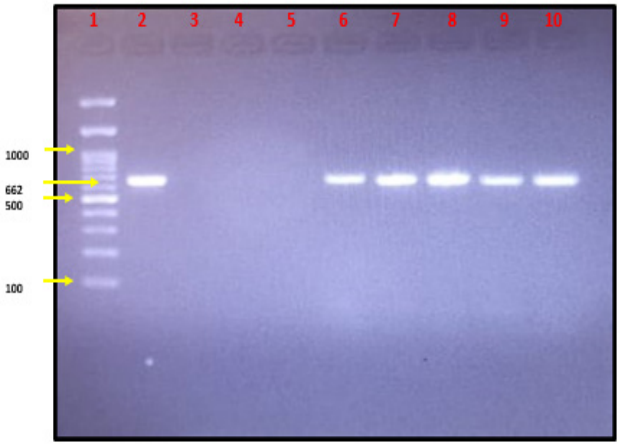

Fig. 2: Screening of blood samples using PCR: Lane 1 100bp DNA ladder, Lane 2 Positive control, Lane 3 Negative control, Lane 4 No template control, Lane 5 Negative sample, and Lane 6, 7, 8, 9 and 10 Positive samples.

Haematological Changes

The mean ± SE values of various haematological parameters of 18 dogs with IMHA caused by *B. gibsoni* and those of 6 healthy dogs are depicted in Table 1. The mean ± SE value of total leucocyte count in dogs affected with IMHA caused

by *B. gibsoni* was significantly higher as compared to the apparently healthy dogs in the control group, while the mean values of Hb, PCV, TEC and platelet count were significantly ($p<0.01$) lower in dogs affected with IMHA as compared to the control group.

Table 1: Mean ± SE of haematological values of apparently healthy dogs (control group) and dogs positive for IMHA caused by *B. gibsoni*

Parameters	Control group of dogs (n=6)	*B. gibsoni* infected dogs with IMHA (n=18)
Haemoglobin (g/dL)	14.47 ± 1.083	5.46 ± 0.266**
Packed cell volume (%)	48.05 ± 2.525	17.27 ± 0.856**
TEC ($\times10^6$/µL)	6.805 ± 0.397	2.178 ± 0.152**
TLC ($\times10^3$/µL)	12.13 ± 0.885	24.04 ± 2.755*
Platelet count ($\times10^3$/µL)	265.2 ± 19.19	66.67 ± 12.27**

*significant at $p<0.05$; **significant at $p<0.01$

A significant reduction in TEC observed in infected dogs concurred with the reports of Yao *et al*. (2014), Brahma *et al*. (2019) and Anju *et al.* (2022). The reduction in TEC in dogs affected with IMHA could be due to antibody mediated cytotoxic destruction of erythrocytes and by antierythrocytic antibodies directed against membranes of infected and uninfected red blood cells (Aysul *et al*., 2013). It could also be attributed to erythrocyte oxidation, osmotic fragility of erythrocytes, a haemolytic factor in the serum, and intra- and extra-vascular haemolysis (Zygner *et al*., 2023).

In the present study, a significant increase in mean TLC value observed in dogs affected with IMHA (Table 1) was in agreement with reports of Jain *et al*. (2017) and Liu *et al*. (2022). Leukocytosis in secondary IMHA may result from tissue necrosis as a result of anaemic hypoxia and thromboembolic condition or it could be attributed to inflammation, sepsis or stress response (Ashwini *et al.,* 2017).

The present study revealed a significant decrease of haemoglobin in dogs affected with IMHA as compared to the healthy dogs, which was in agreement with Yao *et al*. (2014), Anju *et al*. (2022) and Lucy *et al.* (2022). A significant decrease in haemoglobin observed in the present study in IMHA dogs is justified, since IMHA is predominantly a hypersensitivity type II immune reaction in which the immune system produces auto-antibodies that bind to the host's own erythrocytes, triggering both intravascular and extravascular haemolysis (Swann and Skelly, 2013). Anaemia could also occur as a result of increased erythrocytic osmotic fragility, erythrophagocytosis, immune mediated destruction as a result of parasitic antigens, parasite-induced membrane damage and other membrane associated antigens, oxidative damage, sludging and sequestration of erythrocytes (Preena *et al*., 2021).

The present study revealed decrease in PCV in infected dogs which was in agreement with Yao *et al*. (2014). The decreased mean PCV value could be attributed to haemolytic anaemia, intra-vascular and extra-vascular haemolysis and progressive anaemia.

In the present study, a significant decrease in the mean platelet count observed in dogs affected with IMHA caused by *B. gibsoni* compared to control group was in line with Yao *et al.* (2014), Jain *et al*. (2017), and Thomas *et al.* (2019). The reason for thrombocytopenia could be attributed to the platelet sequestration in the spleen, development of disseminated intravascular coagulation and immune mediated destruction of platelets (Reddy *et al*., 2014).

Biochemical Changes

The results of various biochemical parameters of 6 healthy dogs and 18 dogs with IMHA caused by *B. gibsoni* are depicted in Table 2. The mean ± SE values of serum ALT, ALP, BUN, creatinine, and total bilirubin in dogs affected with IMHA caused by *B. gibsoni* were significantly higher when compared with control group, whereas the serum total protein was non-significantly lower, albumin significantly lower, and globulin was non-significantly higher in dogs affected with IMHA than the healthy control group.

Table 2: Mean ± SE of serum biochemistry values of apparently healthy (control group) dogs and dogs positive for IMHA caused by *B. gibsoni*

Parameters	Control group (n=6)	Babesia infected dogs (n=18)
ALT (U/L)	30.27 ± 3.718	71.05 ± 3.200 **
ALP(IU/L)	114.8 ± 15.23	340 ± 30.93 **
Creatinine (mg/dL)	0.93 ± 0.033	1.70 ± 0.075**
BUN (mg/dL)	14.72 ± 2.324	30.62 ± 1.701**
Total protein (g/ dL)	6.25 ± 0.283	5.70 ± 0.142^{NS}
Albumin(g/dL)	2.60 ± 0.109	1.56 ± 0.087 **
Globulin(g/dL)	3.65 ± 0.338	4.13 ± 0.136^{NS}
Total Bilirubin (mg/dL)	0.48 ± 0.054	1.216 ± 0.197*

*significant at $p<0.05$; **significant at $p<0.01$, NS non-significant

Alanine Amino-Transferase (ALT) and Alkaline Phosphatase (ALP)

The present study showed that dogs affected with IMHA caused by *B. gibsoni* had significant increase in the mean ALT and ALP values as compared to control group (Table 2). Significant increase in ALT was observed by Lucy *et al*. (2022). The elevation of ALT in IMHA could be attributed to hypoxic damage of liver (Lucy *et al.,* 2022). It might also be due to escape of the enzyme from the damaged hepatic parenchymal cells with necrosis or altered membrane permeability indicating hepatic dysfunction (Bilwal *et al*., 2017).

Further, a significant increase in ALP activity in dogs with IMHA was in accordance with Thomas *et al*. (2019), and it could be attributed to damage or abnormal function of biliary system (Thomas *et al.,* 2019). A relatively lower difference found in affected and healthy dog by Thomas *et al.* (2019) was attributed to the initial stage of *B. gibsoni* infection in which liver is not subjected to hypoxic changes of the disease.

Creatinine and BUN

A significant increase in the mean values of serum creatinine and BUN observed in dogs affected with IMHA (Table 2), was in accordance with Mittal *et al.* (2019), and Halder and Gupta (2022). The elevation in creatinine could be due to hypovolemia caused due to dehydration. Increased BUN and creatinine in infected dogs could be attributed to acute renal failure (Halder and Gupta, 2022). The elevation could be attributed to haemolysis of RBCs and gastrointestinal haemorrhage. Decreased renal excretion of urea is the most common cause of increased BUN and could be due to decreased blood flow.

Total Protein, Albumin, Globulin

In the present study, a non-significant decrease in total protein and significant decrease in albumin level in dogs with IMHA was observed compared to apparently healthy dogs. These findings were in accordance with Kumar and Kumar (2020) and Anju *et al.* (2022). Albumin is mainly synthesised in the liver. *B. gibsoni* organisms can cause disruption in the liver function that leads to decrease in albumin synthesis and consequently affect total protein levels. Hypoproteinaemia could occur following hypoalbuminemia or protein loss due to haemorrhage which resulted from hypercoagulable states (Ishihara *et al.,* 2010), or due to the disruption in the hepatic protein metabolism, marked decline in diet intake, malabsorption and ongoing protein losing enteropathies like gastroenteritis, gastrointestinal ulcerations and chronic gastritis (Lakshmi and Padmaja, 2021). In the present study there was a non-significant increase in the globulin level in dogs affected with IMHA which was in agreement with Anju *et al*. (2022), who attributed it to increase in immunoglobulins as a response to IMHA caused by *B. gibsoni.*

Total Bilirubin

The present study revealed a significant increase in total bilirubin in dogs affected with IMHA compared to control group (Table 2), which concurred with Vishnurahav *et al*. (2017). The elevated total bilirubin could be due to the intravascular and extravascular haemolysis, progressive anaemia and haemoglobinemia (Jadhav *et al*., 2011). Elevated total bilirubin could also be due to hepatic hypoxia (Vishnurahav *et al*., 2017). Hyperbilirubinemia could be attributed to the damage of the hepatocytes or obstruction of biliary tract associated with inflammation of hepatic parenchyma.

Conclusions

The major haematological changes in IMHA caused by *B. gibsoni* in dogs were significant increase in TLC and a significant decrease in mean TEC, Hb, PCV and platelet count. The major biochemical changes were significant increase in mean ALT, ALP, total bilirubin, creatinine, BUN, significant decrease in mean albumin level and non-significant decrease in total protein with non-significant elevation in globulin levels.

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