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Haemato-biochemical changes associated with Babesia gibsoni infection in dogs

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Abstract

The present study was conducted in dogs presented to the UVH, Kokkalai and TVCC, Mannuthy with clinical signs of inappetence, pyrexia, pale mucous membrane and lethargy suggestive of babesiosis. Forty-five dogs which were primarily screened for B. gibsoni infection through blood smear examination and then confirmed by PCR were included in the study and were subjected to a detailed physical examination, haematology and serum biochemistry analyses. Clinical outcome and variation in haematological and serum biochemical parameters were compared with values of normal dogs. Haematological analysis of affected dogs revealed normocytic normochromic anaemia, mild to moderate thrombocytopenia, inconsistent leucocyte count and differential leucocyte count revealed mild lymphocytosis and granulocytosis. Serum biochemical analysis revealed elevated alkaline phosphatase level.

Keywords: *Dogs, anaemia, Babesia gibsoni, thrombocytopenia.*

Babesiosis is an important canine tick-borne haemoprotozoal disease caused by *Babesia gibsoni* (small *Babesia*) and *Babesia canis* (large *Babesia*) (Preena *et al*., 2019). The severity of the disease may vary depending on the species of babesia infected and the immune response of the host. Immune-mediated haemolytic anaemia and severe inflammatory response syndrome were found to be much related to the varying degree of clinical signs in *B. gibsoni* infected dogs (Parvathy *et al*., 2019).

The clinical signs of *Babesia* infection in dogs may vary from anorexia, pyrexia, malaise, anaemia, icterus, splenomegaly and haemoglobinuria to systemic inflammatory response (SIRS) leading to multiple organ dysfunction (Gonde *et al*., 2016). The study was conducted to analyse the haemato-biochemical changes associated with *B. gibsoni* infection in dogs.

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Materials and methods

Forty five dogs presented to the University Veterinary Hospital, Kokkalai, Thrissur and Teaching Veterinary Clinical Complex, Mannuthy, Thrissur with clinical signs of inappetence, pyrexia, anaemia, pale mucous membrane, haemoglobinuria, generalised lymphadenopathy, lethargy and weakness suggestive of babesiosis were included in the study. Age of the dogs varied from eight months to seven years and included both male and female animals belonging to different breeds including non-descripts. Peripheral blood smears of these animals were examined using Giemsa staining technique. Confirmatory diagnosis was made using polymerase chain reaction (PCR). Six apparently healthy animals were taken as control group to obtain normal values of the parameters under study.

Polymerase chain reaction

Species specific primer pair PIRO- F (5'-AGTCATATGCTTGTCTCA-3') and PIRO-R (5'CCATCATTCCAATTACAA-3') (Trap *et al*., 2006) was used to amplify a 460 bp fragment of the 18S ribosomal RNA gene of *B. gibsoni.* The thermal cycling conditions for DNA amplification is given in table 1.

Haematological analysis

Two millilitres of blood was aseptically collected from the medial cephalic vein or saphenous veinof each dog and transferred to EDTA (HiMedia) coated tube. Complete blood count including the parameters haemoglobin (g/dL), volume of packed red cells (%), total erythrocyte count (TEC) (10^6/mm^3), total leukocyte count (TLC) (10^3/mm^3), differential leukocyte count (per cent), mean corpuscular volume (MCV) (fL), mean corpuscular haemoglobin (MCH) (pg), mean corpuscular haemoglobin concentration (MCHC) (g/dL) and thrombocyte count (10^3/µL) was done using automatic haematological analyzer (Orphee, Mythic Vet 18*)* employing standard technique described by Feldman *et al.* (2000).

Serum biochemical analysis

Aseptically collected two millilitres of blood from the medial cephalic vein or saphenous vein of each dog was transferred to vials containing clot activator (Hi Media, Mumbai). Serum was separated and stored in sterile micro-centrifuge tubes at – 20ºC. Serum levels of creatinine (mg/dL), blood urea nitrogen (mg/dL), alkaline phosphatase (IU/L), total protein and albumin were estimated using commercially available kits (Spinreact, Spain). The assays were performed in a semiautomatic analyzer (Erba Manheim, Chem-5 Plus v2, USA). The ratio of albumin to globulin (A/G) was estimated from the values obtained for the respective biochemical parameters i.e., serum albumin and globulin.

Result and discussion

Examination of the Giemsa stained peripheral blood smears from forty five dogs with clinical signs of fever, lymphadenopathy, anorexia *etc*. revealed intra-erythrocytic piroplasm of *B. gibsoni* organisms which were either single or multiple and signet-ring shaped (Fig.1) (Soulsby, 1982).

Blood samples collected from dogs screened for *B. gibsoni* by blood smear examination were subjected to species specific PCR targeting 18S rDNA gene and yielded 460bp amplicons in 45 clinical samples which were positive for *B. gibsoni* in preliminary blood smear examination (Fig. 2). Polymerase chain reaction was able to detect *B. gibsoni* infection with low parasitaemia (Inokuma *et al*., 2005).

The mean values of various haematological parameters of dogs infected with *B. gibsoni* which was confirmed with PCR are shown in Table 2. Haematological study revealed a significant low level of haemoglobin, VRPC, total erythrocytes and platelet count in *B. gibsoni* infected dogs (Jain *et al*., 2017). Anaemia was one of the most common finding in dogs with canine babesiosis (Vishnurahav *et al.,* 2014). Low erythrocyte count may be associated with multifactorial etiology such as direct mechanical damage of erythrocytic membrane by the parasites, splenic removal of the parasitised RBCs and immune mediated destruction of erythrocytes (Meinkoth *et al*., 2002). Erythrocyte oxidation, anti- erythrocyte antibodies, osmotic fragility *etc*. may also

Table 1. PCR conditions for *Babesia* spp. DNA amplification

	PCR Programme	Temperature (°C)	Time	Cycles
Amplification of 460 bp fragment of 18S rRNA sequence of the *B. gibsoni*	Initial denaturation	95	10 min	
	Denaturation	95	45 sec	40 cycles
	Annealing	52	45 sec	
	Extension	72	1 min	
	Final extension	72	10 min	

Table 2. Haematological parameters of dogs infected with *B. gibsoni*

Hematological parameters	Mean Values ± SD		T– value	p– value
	Infected group (n = 45)	Control(n = 8)		
Total Erythrocyte Count (x 10^6/mm^3)	3.12±1.09	6.69±0.62	-8.397**	< 0.001
Hemoglobin (g/dL)	6.50±2.21	14.68±2.13	-9.144**	<0.001
VPRC (per cent)	20.28±6.7	44.27±6.36	-8.863**	<0.001
MCV (fL)	65.38±4.49	66.12±5.37	-0.396ns	0.693
MCH (pg)	20.95±1.67	21.64±1.45	-1.031ns	0.307
MCHC (g/dL)	31.9±2.15	33.1±2.31	-0.834ns	0.831
Platelet (x 10^3/µl)	125.93±37.69	307.71±65.53	-10.648**	<0.001
Total leucocyte count (x 10^3/mm^3)	13.87±4.71	10.50±3.18	1.822ns	0.074
Granulocyte (per cent)	9.46±4.67	6.20±1.83	1.809*	<0.005
Lymphocytes (per cent)	3.98±1.38	3.02±1.30	0.281ns	0.780
Monocytes(per cent)	0.9256±0.37	0.96±0.31	–0.278 ns	0.782
** - Highly significant (P < 0.01), * - Significant (P < 0.05), ns - Non - significant				

Table 3. Serum biochemical parameters of dogs infected with *B. gibsoni*

Serum Biochemicalparameters	Mean Values ± SD		T– value	p– value
	Infected group (n = 45)	Control(n = 8)		
Creatinine (mg/ dL)	1.11± 0.34	1.06± 0.15	0.361ns	0.720
Blood Urea Nitrogen (mg/dL)	20.10± 10.54	15.63± 3.39	1.104ns	0.275
Alkaline Phosphatase (U/L)	132.15± 168.24	45.14± 18.31	1.356*	<0.05
Total protein (g/dL)	5.95± 0.94	6.41± 0.53	-1.263ns	0.212
Serum Albumin (g/dL)	3.19± 0.55	3.57± 0.34	-1.721ns	0.091
Serum Globulin (g/dL)	3.03± 0.95	2.84± 0.25	0.524ns	0.602
Albumin: Globulin Ratio (A: G)	1.13± 0.33	1.21± 0.11	–0.616ns	0.541
** - Highly significant (P < 0.01), * - Significant (P < 0.05), ns - Non - significant				

contribute to the damage of erythrocytes (Reddy *et al*., 2016). Mean values of both MCV and MCH were found to be lower than that of controlgroup but were within the normal range indicating normocytic normochromic anaemia. In acute infections, bone marrow takes 3 to 5 days to respond to the red blood cell damage and thus the blood picture may reflect non-regenerative anaemia (Schoeman, 2009).

Inconsistent leucocyte counts were also observed in dogs with *B. gibsoni* infection in the study. The differential leucocyte count revealed mild lymphocytosis. This could be due to the enhanced immune response of the body (Vishnurahav *et al.,* 2014) suggesting a chronic or prolonged infection with *B. gibsoni*. Surprisingly, leucopenia was observed in seven *B. gibsoni* infected dogs in the study. According to Rafaj *et al*. (2013), leucopenia in *B. gibsoni* infected dogs could be due to the ability of platelet to bind with the endothelial cells, a further interaction with leucocytes takes place and the so called secondary capture would be induced resulting in the lowering of leucocytes.

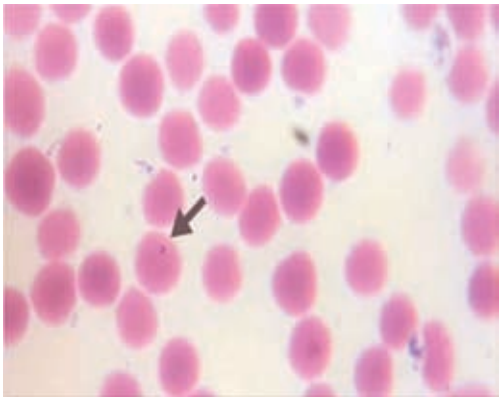

Fig.1. Signet ring shaped *Babesia gibsoni* within the erythrocyte of dog. (Giemsa staining X1000)

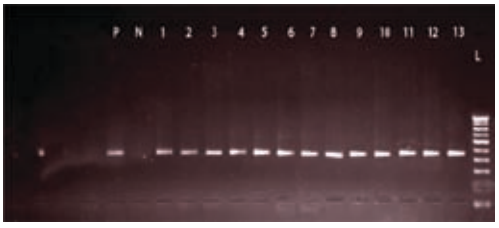

Fig. 2. Agarose gel electrophoresis of PCR products of *B. gibsoni*

L – 100bp DNAladder , P –Positive control, N – Negative control, Lane 1 to 13 – Positive samples of *B. gibsoni*

A moderate to severe thrombocytopenia was observed in the present study. Similar incidence of varying degrees of thrombocytopenia has also been reported by various other authors (Thomas *et al*., 2019; Preena *et al*., 2019). Thrombocytopenia was the most common finding observed in *B. gibsoni* infection and was persisted even after the anaemia was resolved as opined by Meinkoth *et al*. (2002). Though thrombocytopenia was a consistent finding in every animal presented with *B. gibsoni* in the study spontaneous bleeding was not observed in any of the animals, which could be due to the functional activity of thrombocytes (Kettner *et al.,* 2003).

The mean values of serum biochemical parameters of *B. gibsoni* infected dogs are shown in Table 3. On analysis of serum biochemistry, alkaline phosphatase seems to be higher in *B. gibsoni* infected dogs while total protein, albumin level, A:G ratio, creatinine and BUN remained unaltered. This is in accordance with the findings of Thomas *et al*. (2019). To the contrary, significant variations in A:G ratio, albumin, globulin, ALT and GGT with severe organ impairment were recorded in animals with complicated babesiosis by Thankachan *et al.* (2020). The elevated serum biochemical values are mostly associated with the organ failure in *B. gibsoni* infection.

Except for one animal presented with renal involvement which showed an elevated level of BUN and creatinine, all the animals were observed with an unaltered creatinine value. The disproportionate rise in the value of blood urea nitrogen might be associated with the increasedcatabolism of red blood cells that adds to the ammonia load (De scally *et al*., 2004). Vishnurahav *et al*. (2014) and Parvathy *et al*. (2019) observed an elevated globulin levels in *B. gibsoni* infected animals. However, no such observations were made in the present study. The alterations observed in the serum biochemical values were mostly observed in complicated babesiosis that had an involvement of two or more organs. Majority of the animals presented with *B. gibsoni* infection were without any organ impairments.

Conclusion

Deprived appetite, pyrexia, pallor of mucous membrane, lethargy, diarrhoea, melena, lymphadenopathy, jaundice, vomiting, haemoglobinuria and seizures were the major clinical signs observed in dogs infected with *B. gibsoni*. Haematological analysis of affected dogs revealed normocytic normochromic anaemia, moderate to severe thrombocytopenia and inconsistent leucocytes count with mild lymphocytosis while serum biochemical analysis revealed elevated levels of alkaline phosphatase compared to the control group.

Conflicts of interest

There are no conflicts of interest reported by the authors.

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