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Haematological analysis of *Babesia gibsoni* infected dogs

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Abstract
Dogs presented to the University Veterinary Hospitals, Mannuthy and Kokkalai from different parts of Kerala with clinical signs suggestive of babesiosis *viz.*, weakness, anorexia, pallor of mucous membranes and fever were formed the materials for the present study. Animals were subjected to blood smear and representative samples were collected for semi- nested PCR. Twenty four dogs positive for *B. gibsoni* by PCR and blood smear examination were selected for the study. Haematological analysis of affected dogs revealed macrocytic normochromic regenerative anaemia, anisocytosis, thrombocytopenia, leucocytosis with lymphocytopenia and monocytosis.

Keywords: *Babesia gibsoni*, anorexia, clinical signs

Introduction
Canine babesiosis is a tick-borne disease caused by the haemoprotozoan parasites of the genus Babesia with worldwide distribution and global significance. It is considered as an important emerging disease in our country, mainly due to increased transport of pets and climate change. Historically Babesia species have been divided into large (*Babesia canis*) and small (*Babesia gibsoni*) piroplasms. In Kerala both *B. canis* and *B. gibsoni* were first reported from Thrissur district.

The clinical severity of canine babesiosis is variable, and is determined by the Babesia species and the immune response of the host. The two main pathophysiological mechanisms considered to be responsible for clinical signs are haemolytic anaemia, primarily of immune-mediated origin and severe inflammatory response syndrome.

Dogs presented to the University Veterinary Hospitals, Mannuthy and Kokkalai from different parts of Kerala during the period of May 2018 to April, 2019 with clinical signs suggestive of babesiosis *viz.*, weakness, anorexia, pallor of mucous membranes and fever were formed the materials for the present study.

Materials and Methods
Dogs presented to the University Veterinary Hospitals, Mannuthy and Kokkalai from different parts of Kerala with clinical signs suggestive of babesiosis *viz.*, weakness, anorexia, pallor of mucous membranes, fever and jaundice were subjected to blood smear examination and representative samples were collected for semi-nested PCR. Twenty four dogs both blood smear and PCR positive for babesiosis were selected and were subjected to haematological analysis on the day of presentation. Six apparently healthy animals brought to the hospital for vaccination and health checkup were taken as control group to obtain normal values of parameters under study.

About two millilitres of blood was collected in a clean, dry, test tube with EDTA di potassium salt @1mg/ml of blood as anticoagulant for haemogram, leucogram and platelet counts using standard technique as described by Feldman *et al*. (2000) [5]. The following parameters were observed.

1. Haemoglobin (Hb) (g/dL)
2. RBC count (x 106 /mm3)
3. Volume of packed red cells (VPRC) (%)
4. Total leucocyte count (TLC) (X103 /mm3)
5. Differential leucocyte count (DLC) (%)
6. Platelet count (x103 / mm3)

7. Mean Corpuscular Cell Volume (MCV) (fl)
8. Mean Corpuscular Haemoglobin (MCH) (pg)
9. Mean Corpuscular Haemoglobin Concentration (MCHC) (%)

Data obtained were analyzed by using one way analysis of variance (Anova).

Results and Discussion

Giemsa stained peripheral blood smears revealed pleomorphic *B. gibsoni* organisms that appeared mostly as signet-ring shaped inside the erythrocytes and measured 1.2 to 1.9 x 0.7 to 1.1 µm (1.4 x 0.8 µm) (Fig.1) (Soulsby, 1982) [14].

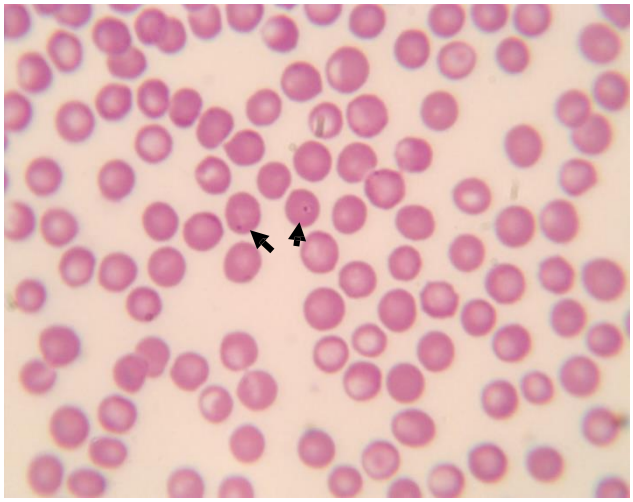

Fig 1: Signet-ring shaped *B. gibsoni* organisms on blood smear examination

Blood samples were subjected to PCR analysis by using genus specific outer primer pair's 455-479F, 793-772R which revealed a fragment between 300 and 400 bp (expected product size was 340 bp) amplified product (Fig. 2).

The amplicons of the genus specific PCR were used as template in a semi nested PCR reaction using *B. gibsoni* species specific primer, BgibAsia- F revealed a fragment between 100 and 200 bp (expected size was 183 bp) which was considered confirmatory for *B. gibsoni* (Fig. 3).

Sequencing of 183 bp PCR product revealed that the amplified product was from a region of 18S rRNA gene. The sequence obtained when analysed using BLAST revealed 99.12 per cent homology with a query coverage of 98 per cent with the published *B. gibsoni* (isolate, New York dog) gene sequence.

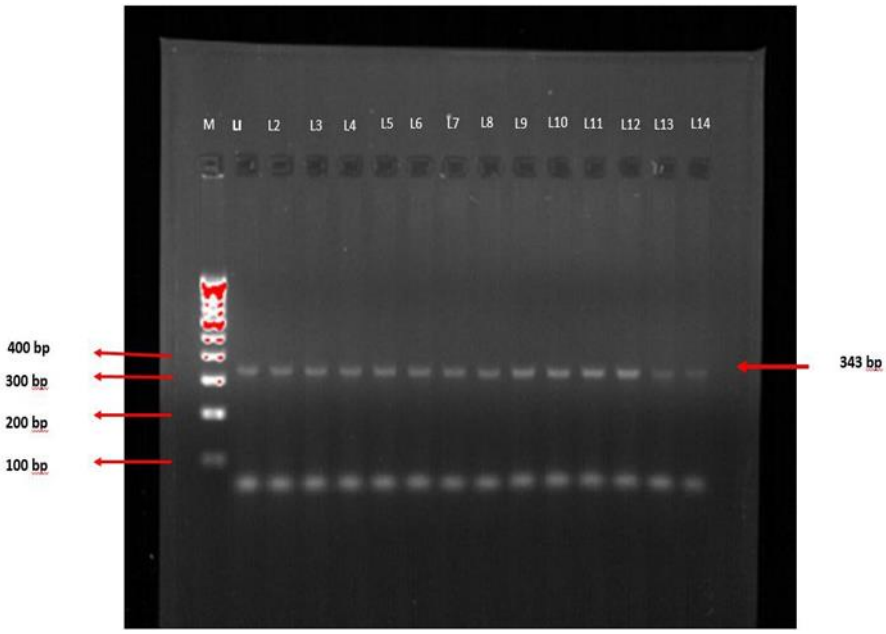

Fig 2: Agarose gel showing PCR amplified product generated with genus specific primers.
M - 100 bp ladder
L1 - L14 amplified product having a size between 300

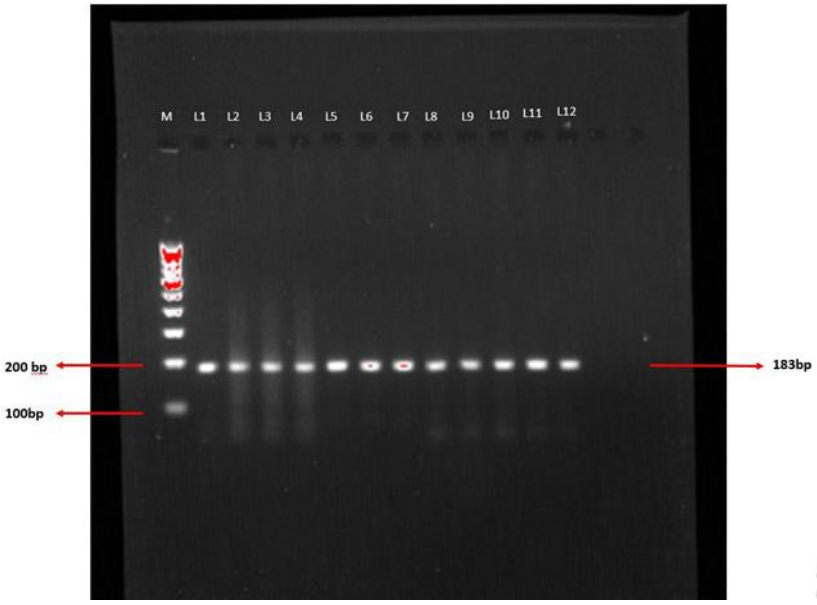

Fig 3: Agarose gel showing semi nested PCR amplified product generated with species specific primer.
M-100 bp ladder
L1-L12 amplified product having a size between 100 to 200 bp

The mean values of haematological parameters of *B. gibsoni* infected dogs were depicted in Table 1.

The study revealed a significant decrease in erythrocyte count, haemoglobin and PCV of *B. gibsoni* infected dogs with macrocytic normochromic regenerative aneamia and anisocytosis. Haemolytic anaemia occurred due to direct parasite induced red blood cell damage, increased osmotic fragility of infected red blood cells, oxidative and secondary immune mediated damage of erythrocyte membrane leading to a combination of intravascular and extravascular haemolysis as opined by Irwin (2009) [6]. The various mechanisms leads to red blood cell lysis were the synthesis of serum haemolytic factors (Onishi *et al*., 1990) [11], antibody binding to cell surface and complement activation (Adachi *et al*., 1992) [2], formation of spherocytes and loss of osmotic fragility of erythrocytes (Makinde and Bobade, 1994) [7], oxidative damage and subsequent phagocytosis of erythrocytes (Murase *et al*., 1996) [10].

Table 1: Haemogram of control and diseased animals on the day of presentation

Haematological parameters	Control animals n=6	Diseased animals n=24	F-value	p-value
Erythrocyte count (x106/µL)	6.38 ± 0.16	2.42 ± 0.22	3.77**	0.00
Haemoglobin (g/dL)	13.30 ± 0.61	5.16 ± 0.47	0.48**	0.00
PCV (%)	36.05 ± 0.78	16.75 ± 2.35	1.73**	0.00
MCH (pg)	20.93 ± 1.13	21.91 ± 0.76	1.47ns	0.55
MCV (fL)	56.63 ± 1.48	63.48 ± 2.09	3.74ns	0.12
MCHC (%)	36.93 ± 1.69	34.17 ± 1.2	0.18ns	0.29
TLC (x103 /µL)	9.90 ± 0.9	14.33 ± 0.96	2.85*	0.03
Granulocytes (%)	55.65 ± 4.24	61.93 ± 2.08	0.00ns	0.19
Lymphocytes (%)	37.61 ± 4.64	28.88 ± 1.78	1.42*	0.04
Monocytes (%)	6.73 ± 0.55	9.22± 0.45	1.84*	0.01
Platelet count (x103/µL)	263.67 ± 14.45	58.96 ± 6.6	0.21**	0.00

*Significant at $p \leq 0.05$ and **- significant at $p \leq 0.01$, ns: non Significant at 0.05

There was statistically significant leucocytosis, lymphocytopenia and monocytosis recorded and the findings were in accordance with Selvaraj *et al*. (2010) [12], who reported moderate leucocytosis in *B. gibsoni* infected dogs. There was marked reduction of lymphocyte blastogenesis and anti-parasite antibody production detected in relapse of clinical *B. gibsoni* infection, leads to immunosuppression (Adachi *et al*., 1993) [1].

Statistically significant thromocytopenia was noticed in diseased group and it might be due to immune mediated destruction of thrombocytes, splenic sequestration or coagulatory consumption of platelets from haemolytic or vascular injury as suggested by Birkenheuer *et al*. (1999) [3] and Solano Gallego and Baneth (2011) [13]. The presence of anti-platelet antibodies in *Babesia gibsoni* infection was detected by flow cytometry by Wlikerson *et al*. (2001) [16]. Thrombocytopenia was considered as a consistent finding in *B. gibsoni* infection and it was detectable and persisted even after the resolution of anaemia as suggested by Meinkoth *et al*. (2002) [9].

Summary

Major clinical signs noticed in *Babesia gibsoni* infected dogs were anorexia, splenomegaly, pallor of mucous membrane, lethargy, fever, lymphadenopathy, jaundice, vomiting, haemoglobinuria and odema of limbs. Haematological analysis of affected dogs revealed macrocytic normochromic regenerative anaemia, anisocytosis, thrombocytopenia, leucocytosis with lymphocytopenia and monocytosis.

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