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Haemato-biochemical alterations and ultrasonographical changes in canines affected with *Babesia gibsoni*

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Abstract
The haemato-biochemical alterations of *B. gibsoni* infection in dogs presented to University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy in Thrissur, Kerala were studied. Seventeen animals with clinical signs of babesiosis which were positive for *B. gibsoni* piroplasms in blood smear were included in the study. Haematological values showed highly significant variations in the values of all the eight parameters; total RBC, total WBC, granulocyte count, monocyte count, MCV, MCH, MCHC and platelet count. Biochemical values showed highly significant variation in A:G ratio, albumin and GGT levels, and significant difference in ALT and globulin levels. The prominent findings of abdominal ultrasonography of animals affected with babesiosis were splenomegaly, nephritis and hepatomegaly. Therapeutic management of dogs affected with B. *gibsoni* should also take into consideration correcting the organ impairment.

Keywords: *B. gibsoni*, haemato-biochemical, ultrasound

Introduction
Canine babesiosis is considered as the most important haemoprotozoan diseases all over the world. Mathe *et al*. (2006) [5] found that *B. gibsoni* was transmitted by ticks *Haemaphysalis bispinosa* and *Haemaphysalis longicornis.* The most common clinical signs noticed during clinical babesiosis were haemoglobinuria, anorexia, fever, lymphadenopathy, pallor and dullness (Aleri *et al*., 2011) [1].
Canine babesiosis is usually perceived just as a haemolytic anaemia by many practicing veterinarians, and treatment mainly targets the etiological agent. Matijatko *et al*. (2010) [6] reported a number of complex manifestations of clinical babesiosis including acute renal failure, coagulopathy, cerebral babesiosis, icterus and hepatopathy. Liver damage and dysfunction was a common finding in the inflammatory stages associated with clinical babesiosis in dogs (Welzl *et al.*, 2001) [9].
This study was undertaken to investigate whether organ impairment was a feature of the canine babesiosis cases presented in the clinical practice, so that appropriate modifications could be made in the therapeutic management protocols.

Materials and Methods
Animals
Dogs presented at TVCC, Mannuthy with clinical signs suggestive of babesiosis like pyrexia, anorexia, inappetance, anaemia, lethargy and haemoglobinuria were included in the study. This included both males and females belonging to different breeds and non-descripts. Age varied from 3 months to 9 years. Signalment of the dogs positive for *B. gibsoni* are given in table 1.

Examination of blood smear
Preparation of thin blood smears
Peripheral blood was collected from the tip of ears into glass slides after clipping and sterilizing of the area with rectified spirit. Five thin blood smears were prepared for each dog, air dried and fixed in methanol for 45 seconds and the slides were stained using Giemsa/Field staining.

Table 1: Signalment of dogs affected with *B. gibsoni*

Sl. No	Breed	Sex	Age
1	Non-Descript	male	6 yr
2	German Shepherd	male	5 yr
3	Non-Descript	male	4 yr
4	Spitz	male	3 yr
5	Labrador Retriever	female	6 yr
6	Labrador Retriever	male	6 yr
7	German Shepherd	female	9 yr
8	Labrador Retriever	male	3 yr
9	Neapolitan Mastiff	male	2.5 yr
10	Dachshund	male	9 yr
11	Pug	male	2 yr
12	Rottweiler	male	1.5 yr
13	Labrador Retriever	female	3 month
14	Rottweiler	male	5 yr
15	Rottweiler	female	2yr
16	Pomeranian	female	6 yr
17	Labrador Retriever	male	4 month

Intra erythrocytic piroplasms of *B. gibsoni* with characteristic signet ring shape were observed under Field's/ Geimsa staining. Other haemoparasitic organisms *Ehrlichia canis*, *Hepatozoon canis* and *Mycoplasma haemocanis* were not noticed in any of these cases.

Haematological analysis

Two ml of blood was collected from the saphenous or medial cephalic vein of each dog under aseptic techniques. The blood was transferred to a collection tube with EDTA (Hi Media, Mumbai). Complete blood count including the parameters total erythrocyte count (TEC) (10^6/µl), total leukocyte count(TLC) (10^3/µl), differential leukocyte count (%), mean corpuscular volume (MCV) (µm^3), mean corpuscular haemoglobin (MCH) (pg), mean corpuscular haemoglobin concentration (MCHC) (g/dl) and thrombocyte count (10^3/ µl) were analysed using an Automatic haematology analyzer (Orphee, Mythic Vet 18).

Serum biochemical analysis

Four ml of blood was collected under aseptic precautions from either the saphenous or medial cephalic vein of each dog. The blood 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, blood urea nitrogen (BUN), alkaline phosphtase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin were estimated using commercially available kits (Spinreact, Spain) and Creatinine kinase-muscle/brain (CK-MB) was estimated using kits procured from Biosystems, Spain. The assays were performed in a semiautomatic analyzer (Erba Manheim, Chem-5 Plus v2, USA). The serum albumin values were subtracted from the corresponding values obtained for total protein in order to estimate the globulin value. 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

Ultrasonography

Ultrasound scanner Esaote MyLab80 was used for the sonographic examination of abdomen of animals. A curvi-linear probe (3mhz – 9 mhz) and a micro convex probe were used. Changes in echogenecity and texture/structure of parenchyma of urinary bladder, spleen, kidneys, stomach, liver and intestines were recorded.

Results and Discussion

Table 2: Clinical signs noticed for dogs affected with *B. gibsoni*

Sl. No	Clinical signs	Number of animals affected
1	Anorexia	17
2	Pyrexia	14
3	Anaemia	12
4	Lethargy	8
5	Vomiting	7
6	Jaundice	5
7	Cystitis	5
8	Respiratory distress	4
9	Limb oedema	2
10	Diarrhoea	2

The statistical analysis of the haematological alterations in dogs affected with *B. gibsoni* are given in table 3. Highly significant variations were noticed in the values of all the eight parameters; total RBC, total WBC, granulocyte count, monocyte count, MCV, MCH, MCHC and platelet count in *B. gibsoni* infections.

Table 3: Statistical analysis of haematological alterations in animals affected with *B. gibsoni* positive dogs

Sl. No	Variable	Mean±SE	Test value	t value	p value
1	Total RBC (x10^6/mm^3)	2.0882 **± 0.17695	5.5	19.281	<0.001
2	Total WBC (x10^3/mm^3)	16.371**±1.7489	6	5.930	<0.001
3	Granulocyte (%)	64.224**±2.6928	51.6	4.688	<0.001
4	Monocyte (%)	8.047**± 0.7220	2.5	7.683	<0.001
5	MCV (fl)	69.082**±1.8051	60	5.032	<0.001
6	MCH (pg)	20.200**± 0.9441	26	6.143	<0.001
7	MCHC (g/dL)	30.029**±1.0247	36	6.827	<0.001
8	Platelets (x10^3/ µl)	81.88**±19.329	200	6.111	<0.001

** - Highly Significant (P< 0.01), * - Significant at (P< 0.05), ns - Non – Significant

The statistical analysis of the biochemical alterations in dogs affected with *B. gibsoni* are given in table 4. Highly significant variation in A:G ratio, albumin and GGT levels, and significant difference in ALT and globulin levels were observed.

Table 4: The statistical analysis of biochemical alterations of dogs affected with *B. gibsoni*

Sl. No	Variable	Mean ± SE	Test value	t value	p value
1	ALP (U/L)	544.529^{ns}±123.5811	320	1.817	.088
2	ALT (U/L)	57.3176*±12.61478	86	2.274	0.037
3	AST (U/L)	72.1118^{ns}±13.38863	54	1.353	.195
4	BUN (mg/dL)	25.84594^{ns}±4.483246	29	0.704	0.492
5	Creatinine (mg/dL)	3.04218^{ns}±1.050062	2	.992	.336
6	Total Protein (g/dL)	8.49788^{ns}±.424018	7.8	1.646	.119
7	A:G ratio	.941718**±.2297493	2.1	5.042	<0.001
8	Albumin (g/dL)	3.22447**±.175448	4	4.420	<0.001
9	Globulin (g/dL)	5.45624*±.471921	4.2	2.662	.017
10	GGT (U/L)	4.7494**±.85758	10	6.123	<0.001
11	CK (U/L)	167.6953^{ns}±69.15020	309	2.043	.058

** - Highly Significant (P< 0.01), * - Significant at (P< 0.05), ns - Non - Significant

Ultrasonography

The sagital, transverse and dorsal views of the viscera were observed. The prominent findings included diffused severe splenomegaly (Fig.1), focal to diffused hepatic architectural variations (Fig. 2), distal acoustic shadowing of gall bladder (Fig.2), loss of cortico-renal distinction and renal architecture (Fig. 3), hyper echogenecity of distal intestinal walls (Fig. 4) as well as cellularity of the contents of the urinary bladder (Fig. 5).

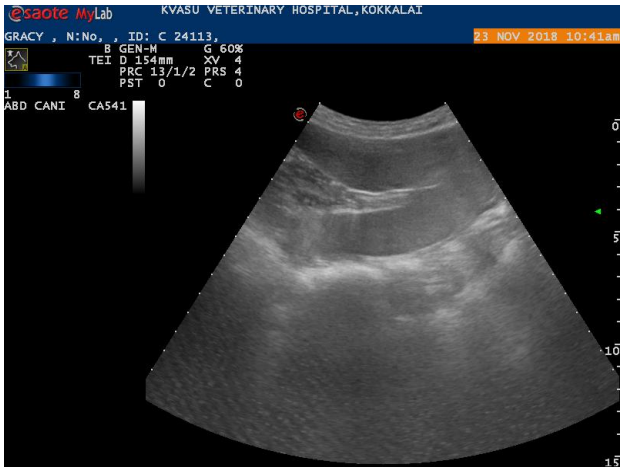

Fig 1: Diffuse splenomegaly with high echogenecity

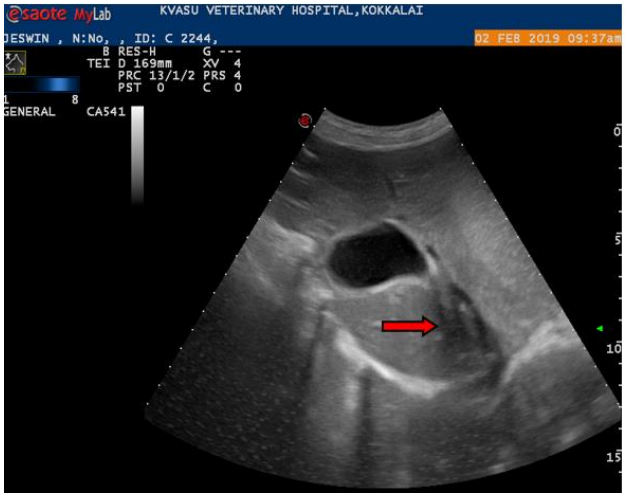

Fig 1: Hepatomegaly with loss of uniform echogenecity, focal and difused inflammatory change

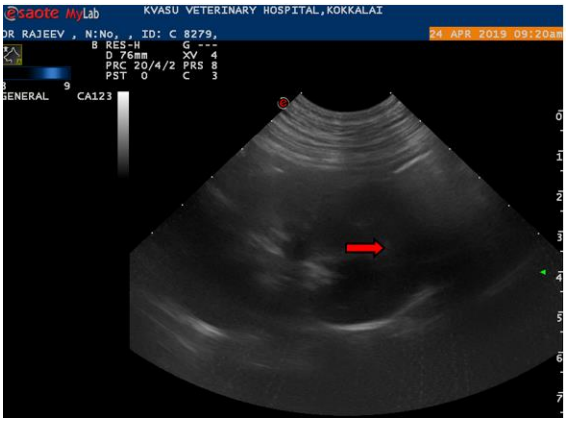

Fig 1: Glomerulo nephritis and chronic inflammatory changes

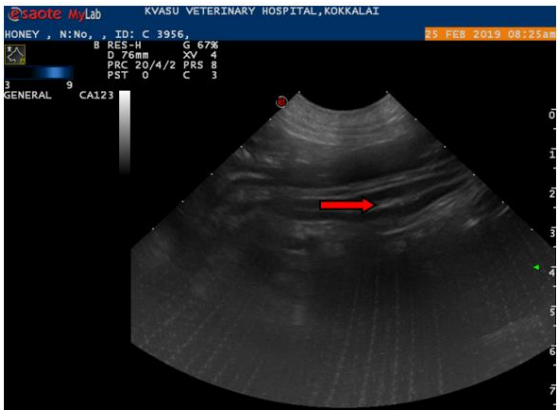

Fig 4: Diffused thickening of intestinal wall with distended loops

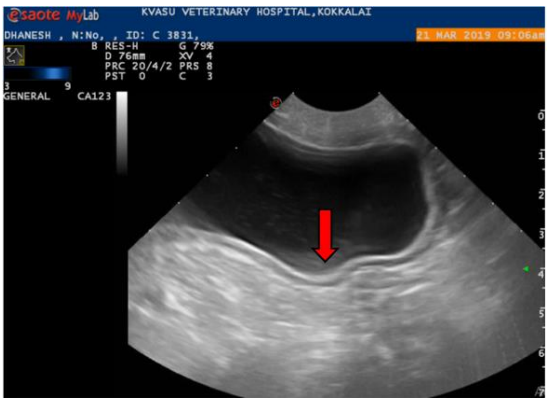

Fig 5: Double rim of the cystic wall with hypoechoic middle

Discussion

Highly significant alterations were evident in all the eight haematological parameters and five out of eight biochemical parameters in the 17 *B. gibsoni* infected dogs. Low RBC, thrombocytopenia, leukocyosis, granulocytosis, high MCV, low MCH and MCHC, typical of haemolytic anaemia were evident. Schoeman *et al*. (2009) had noticed thrombocytopenia in *B. gibsoni* infected dogs, while Furlanello *et al*. (2005) [4] had reported leukopenia in babesiosis affected animals.

Increased levels of ALP, AST, globulin, creatinine and decreased levels of albumin, total protein, BUN, A:G ratio were observed in the animals under study. Impairment of organ function have been reported previously also. Increased ($p< 0.01$) value of alkaline phosphatase, aspartate aminotransferase and globulin as well as decrease in albumin levels ($p< 0.05$) in dogs with babesiosis were observed by Bilwal *et al*. (2017) [2]. Elevated levels of ALT, ALP, Creatinine and BUN were observed by Yogeshpriya *et al*. (2018) [10] who suggested that abnormal function or damage of biliary system resulted in elevated levels of ALP and escape of ALT from damaged hepatic parenchymal cells results in its increased level. Decreased levels of albumin and total protein may be due to liver damage, as reported by Rupali *et al*. (2018) [8]. These observations are in agreement with the findings of this study, as the levels of hepatic enzymes were found to be significantly increased. Hepatic dysfunction was reported by Welzl *et al*. (2001) [9] as a common finding in dogs affected with clinical babesiosis whereas Osterbur *et al*. (2014) [7] cited increase in blood concentration of ALT or ALP, as well as the presence of hepatic encephalopathy as major signs. Observations of abdominal ultrasonography in this study also points to mild to severe alterations in the parenchyma of vital organs, probably as a result of the immune mediated haemolytic anaemia in *B. gibsoni* infections leading to increased release of cytokines ans systemic inflammatory responses. Fraga *et al*. (2011) [3] also observed splenomegaly as the most commonest findings and hepatomegaly and increased cortical echogenicity as frequent findings in babesiosis affected animals.

Conclusion

B. gibsoni infections also involves mild to severe alterations in organ parenchyma and function, which has the potential to affect the outcome of therapy. So, therapeutic protocols should also incorporate efforts to correct or alleviate pathological changes due to this factor for effective recovery of affected animals.

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