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Clinical, haemato-biochemical and ultrasonographical studies on naturally occurring *Babesia gibsoni* infection in dogs

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Abstract
The present study reports the changes in clinical signs, haematology, serum biochemistry and ultrasonographic changes in 8 dogs affected with *Babesia gibsoni* during the month of December 2016 to December 2017. The predominant clinical findings in dogs affected with *B. gibsoni* were icteric mucus membranes, lethargy and pyrexia. Splenomegaly was a common finding in most of the cases. Haematology and serum biochemistry revealed a significant decrease in haemoglobin (7.58125 ±3.71) packed cell volume (20.375±11.12%), total erythrocyte (4.03±1.81×10^6 /µl) and platelet count (43.125±32.18 10^3/µL), significant increase in globulin (4.30625±0.75 gm/dl) in affected dogs when compared to healthy dogs. The naturally occurring cases of *B. gibsoni* are having variety of clinical manifestations ranging from anorexia to hepatomegaly or splenomegaly or death making it difficult to have a definitive diagnosis solely on the basis of clinical examination.

Keywords: *Babesia gibsoni*, splenomegaly, Dogs, Clindamycin

1. Introduction
Canine babesiosis is one of the most important life threatening tick borne haemoprotozoan diseases of dogs caused by intra erythrocytic protozoan parasites of the genus Babesia which are reported worldwide and in various parts of India including Tamil Nadu [1]. Traditionally, canine Babesia has traditionally been recognized as morphologically distinct species, the large *B. canis* and the small *B. gibsoni*. Wide variety of non-specific vague clinical signs [1] was reported with naturally occurring cases of Babesiosis in dogs. *B. gibsoni* is a small pleomorphic intra erythrocytic protozoan parasite reported most commonly than *B. canis*. Due to non-vectoral transmissions of *Babesia gibsoni*, such as blood transfusion and iatrogenic infection, direct blood contact during fighting and biting between dogs; it's gaining importance rather than *B. canis* [2].
Babesiosis caused by *Babesia gibsoni* is less pathogenic and chronic in nature when compared to *Babesia canis*. This verity of babesiosis is related to the extent of parasite replication in the host's red blood cells with subsequent cell lysis [3]. A wide variety of clinical signs like anorexia, lethargy, icterus, vomition and marked loss of body condition have been observed along with variable clinic pathologic abnormalities including haemoglobinuria, hypoglycemia, acid-base disturbances, azotemia, and elevations in the levels of liver enzymes [1]. Further, *B. gibsoni* causes regenerative hemolytic anemia and thrombocytopenia. Hence, the study was conducted to describe various clinical signs and hematobiochemical, ultrasonographical alterations in dogs affected with *B. gibsoni*.

2. Materials and methods
2.1 Case description
The present study was conducted in 32 dogs of various breeds and age groups belonging to both sexes enrolled in Small Animal Referral Unit, Out Patient Ward during a one year study period at Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu from different parts of Delta region of TamilNadu with clinical signs suggestive of babesiosis viz., weakness, anorexia, pallor of mucous membranes, fever and jaundice (Fig.1, 2).

2.2 Parasitological examination
On microscopic examination of Giemsa stained thin blood smears prepared from the ear margin was carried out under oil immersion (100x) lens [4]. Among 32 patients, 8 dogs revealed the presence of ring shaped, oval, parachute, and comma-like organisms in erythrocytes (Fig.3) on examination. One of the most commonly observed form of *Babesia gibsoni* was signet ring shape in erythrocytes [5]. On the basis of the size of the intracellular parasites in this case, the possibility that the dog has been infected with small Babesia spp., especially with *B. gibsoni* was considered.

2.3 Hematological and biochemical examination
Around 5 ml of blood was collected from either the cephalic vein / recurrent tarsal vein in a dry vial containing 10 per cent anticoagulant EDTA for complete hematological studies. Hematological investigations were estimated as per standard methods [6]. Five millilitres of blood were collected in vacutainer without anticoagulant taking all precautions for avoiding hemolysis. Serum was separated and was used for quantitative estimation of total protein, albumin, Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Serum Urea Nitrogen (SUN) and Creatinine [6].

2.4 Ultrasonographic examination
Ultrasonographic examinations were performed without sedation or anesthesia, with the dogs in dorsal and lateral recumbency [7]. After hair clipping and application of alcohol and acoustic coupling gel to the skin, B-mode sonographic evaluation of the liver, spleen, gall bladder, right and left kidneys and prostate was performed using fundamental ultrasonography (Esaote Mylab one) with 5.0 MHz convex and 7.5 to 12.5 MHz linear transducers.

2.5 Statistical analysis
The data obtained were subjected to the statistical analysis as per standard methods [8]. The independent t-test having means with unequal variances was carried out. Variables with $p<0.05$ were considered as statistically "significant," variables with $p<0.01$ were considered as statistically "highly significant" and variables with $p>0.05$ were considered as statistically "non-significant."

3. Results and discussion
Significant reduction in RBC, Hb concentration, PCV percentage and platelet count were recorded among infected dogs when compared to the apparently healthy dogs. Decreased Hb and RBC levels might be due to direct mechanical disruption caused by parasite as it leaves red blood cells, intravascular hemolysis, and immune-mediated or non-immune mediated destruction of red blood cells or due to severe anemia [8]. These organisms initiate a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes. The mechanisms of the thrombocytopenia are not yet fully understood in babesiosis [9]. The reason for thrombocytopenia in babesiosis could be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation. The leukogram changes are nonspecific, although severe transient neutropenia (<1,000/µl) was noted in several dogs [10] one week after experimental infection with *B. gibsoni*.

Serum biochemical parameters revealed increased BUN, Creatinine, ALT, ALP levels were noticed. Haematological and biochemical findings were incorporated in the Table 1. Increase in level of ALP could be due to damage or abnormal function of biliary system. Increased activities of ALT were might be due to escape of these enzymes from the damaged hepatic parenchymal cells with necrosis or altered membrane permeability indicating hepatic dysfunction [11]. Renal involvement in these cases could be due to damage to renal cells caused by inflammatory mediators, or possibly due to the development of refractory hypotension resulting in reduced renal tissue perfusion and glomerular filtration rate [12].

This should be differentially diagnosed from immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, zinc toxicity, rickettsial diseases, bartonellosis, leptospirosis, dirofilariasis with caval syndrome, systemic lupus erythematosus, and neoplasia.

An ultrasonographic pattern consisting of nearly equal, marked increase in cortical and medullary echogenicity and relatively hypoechoic corticomedullary junction and central medullary regions was recognized concurrent with the development of anuria in 3 of the 8 dogs. Mild, transient increases in cortical and medullary echogenicity were observed in 3 dogs. Two dogs had no sonographic abnormalities.

The spleen serves as a major source in the immune defense against babesia infections as it is directly involved in sequestration and destruction of the organisms. Unlike bovine babesiosis, hemoglobinuria is rarely seen in canine babesiosis [13]. Diffuse enlargement of spleen with mild hypoechogenous pattern of parenchyma on ultrasonography (Fig.4, 6) could be the result of diffuse proliferation of lymphocytes and plasma cells in the white and red pulp and non-specific pooling or sequestration of erythrocytes and platelets or due to abundance of hosting macrophages in this organ [13].

Table 1: Haemato-biochemical values of *Babesia gibsoni* infected dogs (Mean ± S.E)

Parameters	Normal healthy dogs (n=8)	*Babesia gibsoni* infected dogs (n=8)
Hb (g/dl)	12.03 ± 0.36	7.58125 ±3.71**
RBC ($\times10^6$/µl)	6.51 ± 0.19	4.03±1.81*
PCV (%)	39.29 ± 2.14	20.375±11.12**
TLC($\times10^3$/µl)	9.33429 ± 8.134	12.91125±4.17
Platelets (10^3/µL)	216.25±18.31	43.125±32.18**
Neutrophils (%)	65.45± 2.18	66.875±6.38
lymphocytes (%)	20.89±2.57	28±6.74
Monocytes (%)	2.45±0.21	3.875±1.81
Esinophils (%)	1.80±0.90	1.5±1.41
Serum Urea Nitrogen (mg/dl)	16.56 ± 0.95	123.5±56.77**
Creatinine (mg/dl)	0.91 ± 0.07	3.65375±1.82**
ALT U/L	18.71 ± 1.56	63.25±23.61*
Total Protein (gm/dl)	6.81 ± 0.22	7.8875±0.75
Albumin (gm/dl)	3.25 ± 0.04	3.58125±0.52
Globulin (gm/dl)	3.56 ± 0.20	4.30625±0.75
A/G ratio	0.85 ± 0.11	0.862803±0.24
Alkaline Phosphatase (U/L)	87.05± 16.31	139.5±34.83*

*$p<0.05$ - significant, **$p<0.01$- highly significant and $p>0.05$ non-significant

Fig 1: Vaginal mucus membrane of dog infected with *B.gibsoni* (Before treatment)

Fig 2: Vaginal mucus membrane of dog infected with *B.gibsoni* (After treatment)

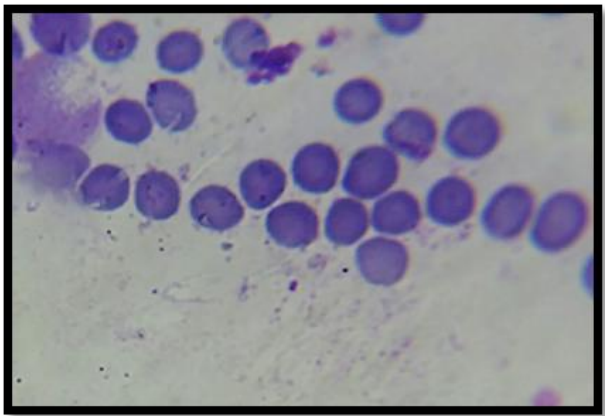

Fig 3: Peripheral blood smear revealed *B. gibsoni* in RBC

Fig 4: Ultrasonogram of enlarged spleen

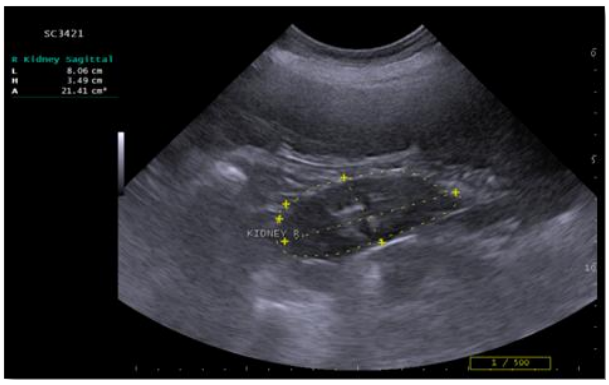

Fig 5: Ultrasonogram of Kidney of B.gibsoni affected dog

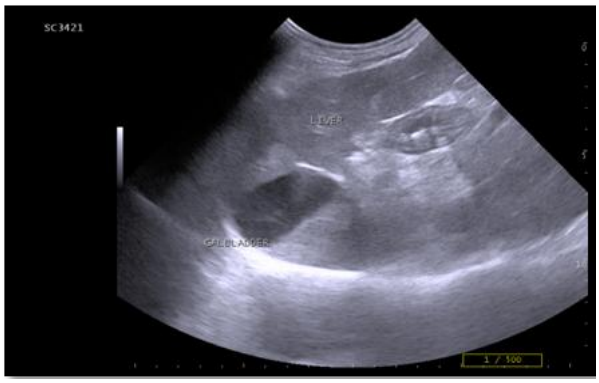

Fig 6: Ultrasonogram of liver and gall filled bladder

4. Conclusion

The present study that *B. gibsoni* had a wide variety of clinical manifestations so dogs showing erratic fever, weight loss, depression, pale mucosa, and splenomegaly alone or in combination can be suspected for babesiosis. As babesiosis is spread through tick, control of the vector tick is essential for prevention of disease. Application of acaricidal/insecticidal with ectoparasiticides and additional repellents reduces the arthropod-host interaction and can thus reduce the risk of infection. Prevention of tick must be an established tool of disease prophylaxis in any dog living in vector endemic areas or traveling with its owner to such regions. Dog owners should be made aware of the risks and the need for protection by their veterinarians.

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