RESEARCH ARTICLE

Clinico-Pathological Evaluation of *Babesia gibsoni* Associated Renal Failure in Dogs

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Abstract

The aim of the current study was to assess clinical, haematological, and biochemical alterations in dogs infected with *Babesia gibsoni*, specifically associated with renal complications. Blood samples were obtained from 44 dogs exhibiting symptoms such as fever, lymph node enlargement, and reduced urine output. *Babesia* piroplasms were detected in 5 cases through blood smear examination, while PCR confirmed 10 positive cases. The dogs that were found positive for *B. gibsoni* underwent a comprehensive haematological and biochemical analysis to assess the severity of their condition. The primary haematological abnormalities observed were anaemia and thrombocytopenia. Furthermore, the serum biochemical analysis revealed elevated levels of BUN and creatinine, indicating renal insufficiency. Post-mortem examination revealed grossly a capsule firmly adhered to the kidney and during histological examination there was renal tubular dilatation and presence of renal fibrosis. In the chronic stage of the disease, despite the absence of antigen replication within the host's body, the initiated inflammatory response can result in damage to multiple body systems. Therefore, it is of utmost importance to assess the disease's advancement and the overall condition of the animal.

Key words: *Babesia gibsoni*, Biochemistry, Haematology, Histopathology, PCR.

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Introduction

The haemoprotozoan parasite, *Babesia gibsoni*, causes economically significant and potentially life-threatening disease to dogs in India (Jacobson *et al*., 1994). *Babesia gibsoni* induces a spectrum of disease severity characterized by hyperacute, acute, and chronic infection stages. During acute and recurrent infections, clinical manifestations typically include pyrexia, reduced appetite, lymphadenopathy, thrombocytopenia, anaemia, elevated liver enzyme levels, and haemoglobinuria. In contrast, chronic infections are characterized by low grade fever, lethargy, icterus, weight loss, hepatomegaly, splenomegaly, and widespread systemic involvement (Miyama *et al*., 2005; Torbica *et al*., 2013; Koster *et al*., 2015).

Acute renal failure (ARF) is an unusual complication of babesiosis which usually manifests as anuria or oliguria in spite of adequate rehydration (Jacobson *et al*., 1994). Renal changes in babesiosis have been attributed to haemoglobinuria and referred to as haemoglobinuric nephropathy. The true pathogenesis of renal lesions in babesiosis is still obscure. The diagnostic strategies for babesiosis span from conventional microscopic examination of blood smears to sophisticated molecular techniques like PCR (Lempereur *et al*., 2017; Wahlang *et al*., 2019). As per the previous retrospective study report, prevalence of canine haemoprotozoan diseases were in an increasing trend in Chennai, with an overall prevalence of 21.77% *B. gibsoni* through blood smear examination (Senthil and Chakravarthi, 2023). Given the systemic implications in chronic cases, it is imperative to assess the disease's severity. Therefore, the purpose of this study was to investigate the clinical and pathological features of canine babesiosis in dogs with renal insufficiency.

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Materials and Methods

Overall 44 canine blood samples from suspected *Babesia* cases with history of ticks, clinical signs of pyrexia, epistaxis, palpable lymph nodes, pale mucus membranes, anaemia, thrombocytopaenia, icterus, which had clinical complications

associated with kidney presented to Critical Care Unit, MVC, Chennai were collected. The preliminary diagnosis of babesiosis was done by identifying piroplasms in the Leishman-Giemsa (LG) cocktail stained blood smear. Genomic DNA was extracted from the suspected blood samples using DNeasy® Blood and Tissue kit (Qiagen). Briefly, whole blood (200 µL) was lysed using buffer AL and proteinase K. The lysate was loaded onto QIAamp mini spin columns followed by washing using AW_1 and AW_2 buffers. Then, the DNA eluted in 100 µL buffer AE was quantified using Nanodrop (Thermo Fischer) and stored at -20 °C till further use. Later, the DNA was subjected to PCR assay using *Babesia gibsoni* species specific primers F, 5′ TGGGGTTTTCCCC TTTTTAC 3′ and R 5′ TCTGGACCTGGTGAGTTTCC 3′ targeting 18S rRNA gene (Satish *et al*., 2021). The amplified PCR products were subjected to agarose gel electrophorosis on ethidium bromide stained 1.5% agarose gel at 100v for 30 min in 1x TAE buffer alongside a 100 bp DNA marker. The results were visualized and documented in gel documentation system.

For the blood samples collected from suspected dogs a complete blood count was performed by automatic haemo-analyser (Auto Haemo Analyser- 4 part- Exigo). The total haemoglobin concentration, erythrocyte count, packed cell volume (PCV), total white blood cell count as well as differential leukocyte count and thrombocyte count were evaluated. Blood samples were also collected in clot activator tubes for biochemical analysis, *viz*., BUN, creatinine, total protein (TP), albumin, bilirubin, alanine amino transferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, glucose and cholesterol using standard kits in an automated clinical chemistry analyser (Auto Biochemistry Analyser - A15). The arithmetical mean and standard deviation were calculated for all parameters using standard statistical tools.

Results and Discussion

On examining the blood smears of 44 blood samples, small pleomorphic forms either with or without pyriform shape were observed in 5 samples suggesting the presence of *B. gibsoni* piroplasms (Fig. 1). For further confirmation, PCR was performed as per the protocol described by Satish *et al*. (2021). The amplified PCR products showed amplification in 10 samples, revealing the desired amplicon size of 489 bp (Fig. 2).

Table 1: Haematological and serum biochemical parameters of dogs with *Babesia gibsoni*

Parameter	Units	Mean ± SE	Observation range	Reference range*
Haematological Parameters				
Hb	g dL^{-1}	6.11±0.97	2.9-11	11.9-18.9
RBC	$\times10^6$ μL^{-1}	2.765±0.45	1.17-5.3	4.95-7.87
PCV	%	17.81±2.91	7.9-29.5	35-57
WBC	$\times10^3$ μL^{-1}	16.16±3.745	3.4-40	5-14.1
Neutrophils	%	77±2.14	69-90	58-85
Lymphocytes	%	17±2.03	6-25	8-21
Monocytes	%	4.9±0.36	4-8	2-10
Eosinophils	%	1.2±0.25	0-2	0-9
Platelets	$\times10^3$ μL^{-1}	36.02±8.48	8.6-70	211-621
Serum Biochemical Parameters				
BUN	mg dL^{-1}	24.61±4.01	8.52-44.71	8-28
Creatinine	mg dL^{-1}	1.47±0.59	0.34-7.16	0.5-1.7
Total protein	g dL^{-1}	6±0.57	1.9-7.9	5.4-7.5
Albumin	g dL^{-1}	2.25±0.11	1.8-2.7	2.3-3.1
Bilirubin	mg dL^{-1}	1.84± 1.04	0.49-8.03	0-0.3
ALT	IU L^{-1}	62.63±10.19	26-138	10-109
ALP	IU L^{-1}	130.98±14.79	75-194	1-114
Calcium	mg dL^{-1}	9.71± 0.38	8.49-11.3	9.1-11.7
Phosphorus	mg dL^{-1}	5.63± 0.89	2.53-10.19	2.9-5.3
Glucose	mg dL^{-1}	91.9±13.61	30-161	76-119
Cholesterol	mg dL^{-1}	145.6±20.63	67-296	135-278

*Reference values were taken from MSD Veterinary Manual.

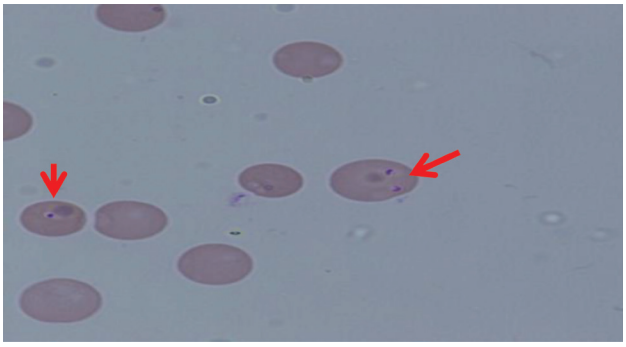

Fig. 1: Blood smear of Babesia gibsoni – Leishman Giemsa (LG) stain x 1000

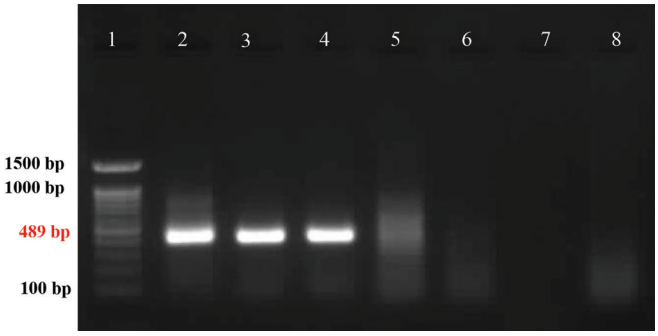

Fig. 2: PCR Screening of *Babesia gibsoni*: Lane 1: 100 bp Ladder lane, 2: *Babesia gibsoni* positive control, 3 & 4: Positive, 5, 6 & 8: Negative, 7- Negative control

Further, a broad spectrum of haematological and serum biochemical findings recorded (Table 1) signified varying degrees of disease severity among the *Babesia gibsoni* infected dogs associated with renal insuffiency.

The majority of the cases of this study had a PCV less than 30% and the infected animals had less haemoglobin concentration and RBC counts indicating anaemia. *Babesia* triggers an immune response involving the antibody-mediated cytotoxic destruction of circulating red blood cells, which results in both intravascular and extravascular haemolysis, leading to onset of anaemia and haemoglobinemia (Irwin, 2005; Shah *et al.,* 2011). All the babesia infected dogs had less platelets count, reflecting severe thrombocytopenia, which appears to be a complex phenomenon with multiple potential contributing factors, including elevated body temperature, immune-driven platelet destruction, platelet sequestration in spleen, and consumption of platelets during the coagulation process (Solano - Gallego and Baneth 2011). Haemolytic anaemia and thrombocytopenia are the primary haematological abnormalities observed in blood parasite infected dogs (Sudhakara *et al.,* 2016; Brahma *et al.,* 2019; Gonmei *et al.,* 2020; Gurjar *et al.,* 2023).

The mean white blood cell count was slightly elevated, and also elevated neutrophils and lymphocytes count in some cases was observed indicating a co-infection or secondary infection. Gonmei *et al.* (2020) reported increased white blood cell count as a consistent change. An increased leukocyte count, specifically neutrophil count is a sign of dog's immune response to infection and inflammation associated with the disease (Selvaraj *et al.,* 2010; Bilwal *et al.,* 2017; Kumar and Kumar,2018).

The serum biochemical results showed elevated BUN and creatinine values representing renal insufficiency of affected dogs in the present study. The serum urea and creatinine levels can be proportionately increased (Sudhakara *et al.,* 2016; Roopali *et al.,* 2018; Gonmei *et al.,* 2020) indicating decreased renal perfusion, possibly as a result of decreased blood pressure, decreased myocardial function, and/or hypovolemia (Koster *et al.,* 2015).

According to the severity of the animal's condition, the mean levels of total protein, albumin, glucose, and cholesterol were observed to decrease in the current investigation. Hypoglycemia is common complication associated with canine babesiosis, potentially arising from factors such as starvation, sepsis causing anorexia and impaired hepatic function (Koster *et al.,* 2015; Sudhakara *et al.,* 2016; Kumar and Kumar, 2018). While there wasn't a substantial difference in ALT levels, the significant increase in bilirubin and ALP levels suggest the presence of liver insufficiency or dysfunction in this investigation. Previously documented findings have indicated elevated ALT, ALP and total bilirubin levels in *Babesia* infected dogs (Bilwal *et al.,* 2017; Kumar and Kumar, 2018). This may be due to damage or abnormal function of biliary system and intravascular or extravascular haemolysis leading to hyperbilirubinemia (Crnogaj *et al.,* 2010; Shah *et al.,* 2011).

A post-mortem examination was performed on a dog that had died due to renal insufficiency associated with *B. gibsoni*. Macroscopic examination of the kidneys disclosed the presence of a firm capsule tightly adhered to the kidney and petechial haemorrhages on the kidney's surface (Fig. 3). Additionally, the urinary bladder contained dark reddish-brown urine. A histopathological assessment of the kidney indicated renal tubular dilatation (Fig. 4) and renal fibrosis (Fig. 5), suggesting that the dog may have been afflicted with Chronic Kidney Disease (CKD). These observed changes were in accordance with findings in prior studies (Lobetti and Jacobson, 2001).

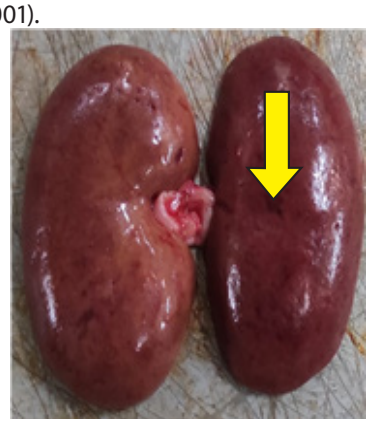

Fig. 3: Kidney with haemorrhages

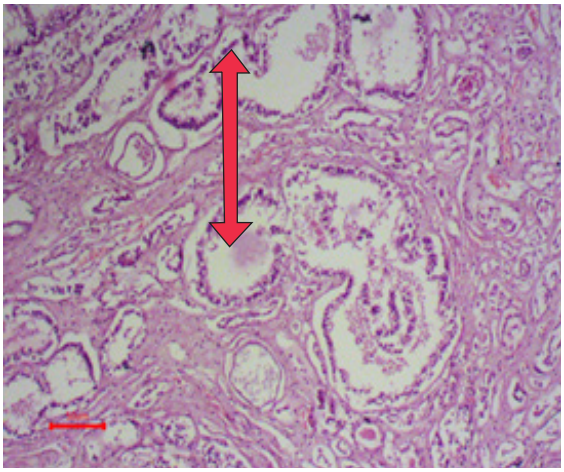

Fig. 4: Renal tubular dilatation

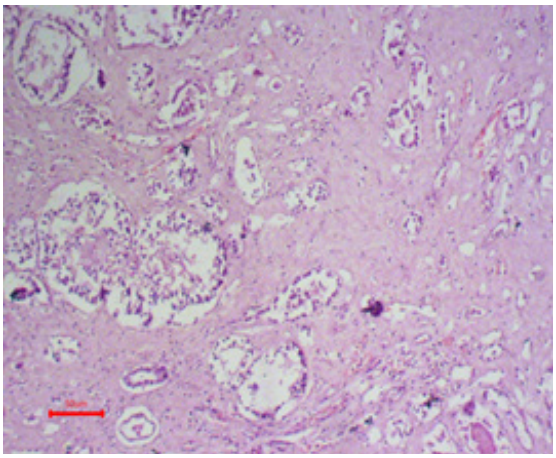

Fig. 5: Renal fibrosis in CKD

Conclusion

The predominant and consistent haematological findings of canine babesiosis associated with renal failure in dogs under study comprised of anaemia and thrombocytopenia, while in terms of serum biochemistry, increased levels of BUN, creatinine, ALP, bilirubin, and hypoglycemia were consistent. The clinical presentation of canine babesiosis can vary from mild to severe. In chronic cases, the infection has the potential to affect multiple body systems, including the kidneys, leading to renal insufficiency. Understanding the clinical and pathological features of babesiosis in dogs with renal insufficiency is crucial. So, regular monitoring of disease's progression, its impact on various organs, and severity of disease is crucial for developing effective treatment strategies and improving the overall management of canine babesiosis, especially in cases with systemic involvement like renal insufficiency.

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