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## Clinical, haematological, and biochemical alterations in dogs affected by babesiosis

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### Abstract

Dogs with fever, inappetence, lymph node enlargement, tick infestation and pale mucus membranes were screened for the haemoprotozoan diseases during the period under study at College of Veterinary Science, Proddatur. This study presents the observed changes in clinical signs, haematology and serum biochemistry of 27 dogs diagnosed with *Babesia* infection from October 2023 and September 2024. Clinical examination revealed inappetence, pyrexia, lymphadenopathy, weakness, lethargy, pale conjunctival/buccal mucus membranes, change in urine colour, congested mucus membranes, dependent edema and icteric mucus membranes. Laboratory examination of the clinical samples revealed reduction in haemoglobin, packed cell volume, total erythrocyte count and platelet count when compared to the apparently healthy dogs. There was significant elevation of alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and creatinine along with significant decrease in the albumin levels in the affected dogs. The colour of the urine was red brown, yellowish orange and yellow in affected dogs while the urinalysis revealed increase in pH and specific gravity of urine, proteinuria and bilirubinuria.

**Keywords:** *Babesia*, biochemistry, clinical observations, dogs, haematology

### Introduction

Canine babesiosis is a clinically significant and geographically widespread haemoprotozoan disease of domesticated dogs and wild canids. The parasite was mainly transmitted through tick vector and also transmitted by blood exchange, dog bite and transplacental route (Jefferies *et al.*, 2007) [9]. In the present study, the clinical signs and haemato-biochemical changes in the particular locality of the animals will provide the individual animal health status, which is further helpful to the assess the involvement of all the visceral organs and useful for the formulation of the therapeutic regimen. (Reddy *et al.*, 2022, Azimzadeh and Mohammadisefat, 2022) [12, 11].

### Materials and Methods

The present study was performed at Department of Veterinary Medicine, College of Veterinary Science, Proddatur from October 2023 to September 2024. In this study, a total number of 620 dogs belongs to the different breeds were examined for the presence of *Babesia* in peripheral blood smears. Among 620 dogs, 136 dogs were found positive for *Babesia* infection, out of which 27 dogs were selected for complete clinical and haemato-biochemical analysis. Peripheral blood was collected from the ear tips of the dogs for Giemsa-stained thin blood smear examination to detect *Babesia* species in red blood cells. The smears were fixed in methanol for 3 mins and stained by 10% Giemsa staining solution (Sivajothi *et al.*, 2022) [15]. Two blood smears were made from each sample and a minimum of 50 fields of each stained blood smear were examined under oil immersion to determine parasitaemia. Blood was collected in EDTA coated vials and used for estimation of haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), total leucocyte count (TLC) and platelet count. The peripheral blood smears were stained by Leishman's stain to study the differential count (DLC) by battlement method following which, the percentage counts were calculated. 5 ml of blood was collected from cephalic vein into vial containing the clot activator for separation of serum and study serum biochemical parameters including blood urea nitrogen, creatinine, alanine amino transferase, total protein, serum

albumin, serum globulin by using Span diagnostics Ltd. Kits. Haematological and biochemical findings of the infected dogs were compared with haemato-biochemical findings of apparently healthy dogs of the local area (Reddy *et al.*, 2016) [13].

## Results and Discussion

Microscopic examination of the peripheral blood smears demonstrated the presence of typical small, oval shaped and pear like structures *Babesia* in the erythrocytes of 136 dogs in this study. Clinical examination of the 27 infected dogs revealed, presence of fever 24/27 (88.88%), ticks over the body 22/27 (81.48%), inappetence 22/27 (81.48%), depressed demeanour 21/27 (77.77%), pale mucus membranes 21/27 (77.77%), lymphadenopathy 19/27 (70.37%), emaciation 17/27 (62.96%), dyspnoea 14/27 (51.85%), tachycardia 14/27 (51.85%), tachypnoea 12/27 (44.44%), nervous signs 11/27 (40.74%), scleral congestion 10/27 (37.03%), icteric mucus membranes 5/27 (18.52%), haemoglobinuria 3/27 (11.11%), dependent oedema 2/27 (7.40%) and hypothermia 2/27 (7.40%) (Table-1).

Haematological parameters were in the Table 2. Significant reduction in haemoglobin concentration ( $9.52 \pm 0.12$  g/dL), red blood cell count ( $4.73 \pm 0.46 \times 10^6/\mu\text{L}$ ), and packed cell volume percentage ( $28.20 \pm 2.51\%$ ) were recorded among infected dogs when compared to the apparently healthy dogs with red blood cell count ( $6.51 \pm 0.19 \times 10^6/\mu\text{L}$ ), haemoglobin concentration ( $13.03 \pm 0.42$  g/dL) and packed cell volume percentage ( $39.29 \pm 2.14\%$ ). Significant decrease in white blood cell count ( $8786.67 \pm 71.45 \times 10^3/\mu\text{L}$ ) and neutrophilia ( $82.91 \pm 2.5\%$ ) along with lymphocytopenia ( $13.03 \pm 1.86\%$ ), eosinophils ( $3.22 \pm 0.27\%$ ) and monocytes ( $2.34 \pm 0.27\%$ ) was noted in dogs with *Babesia* infection compared to apparently healthy dogs there is a significant variation was observed. Dogs with *Babesia* infection had lowered thrombocyte count ( $1.24 \pm 0.21 \times 10^3/\mu\text{L}$ ) than compare the mean count in apparently healthy dogs ( $2.71 \pm 0.28 \times 10^3/\mu\text{L}$ ). Serum-biochemical changes in dogs with babesiosis presented in the Table-3. Reduction in total protein ( $4.48 \pm 0.21$  gm/dL), serum albumin ( $1.23 \pm 0.09$  mg/dL) and increased total bilirubin levels ( $1.12 \pm 0.12$  mg/dL), alanine aminotransferase ( $53.00 \pm 8.2$  U/L) and alkaline phosphatase ( $139.5 \pm 14.83$  U/L), blood urea nitrogen ( $45.67 \pm 2.55$  mg/dL) and creatinine ( $1.57 \pm 0.19$  mg/dL) levels were noticed and when compared apparently healthy dogs there is a significant variation was observed.

Abnormal findings were noticed in three dogs included haemoglobinuria, proteinuria, bilirubinuria, and increased amount of urobilinogen in the urine. Microscopic evaluation of the sediment revealed red blood cells as well as white blood cells, tubular epithelial cells and crystal formation.

## Discussion

Clinically the illness of babesiosis was characterized by fever, pale mucous membrane, loss of appetite, anaemia, icterus and lymphadenopathy (Boozer and Macintire,

2003) [2]. Clinical signs are exceedingly variable; the classical presentation is a febrile illness with perceptible anaemia. The severity of babesiosis varies from sub clinical infection to extensive organ failure and death. The most common hematologic alterations associated with *Babesia canis* infections are anaemia and thrombocytopenia (Kumar and Kumar, 2020) [10]. *Babesia* organisms initiate a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes (Selvaraj *et al.*, 2010) [17]. 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 ( $<1000/\mu\text{L}$ ) was noted in several dogs (Thongsahuan *et al.*, 2020) [18]. Once inside the host *Babesia* organisms are attached to the erythrocyte by endocytosis. Due to the parasites invading and replicating in the erythrocyte, babesiosis results in destruction of the erythrocyte. The destruction of the erythrocyte is multifactorial, including direct parasite damage to the infected erythrocyte and antibody mediated cytotoxic destruction of erythrocytes. The immunological response plays the most important role in pathogenesis of canine babesiosis. Autoantibodies are directed against components of the membranes of infected and uninfected erythrocytes which causes intravascular and extravascular haemolysis (Malyuk *et al.*, 2023) [11]. Anaemia and thrombocytopenia were the most common haematological alterations observed in the present study which concurred with the previous reports (Gonde *et al.*, 2017; Brahma *et al.*, 2019) [6, 3]. Persistent anaemia and thrombocytopenia are common in blood parasite infection in dogs (Russell, 2010) [14]. *Babesia* initiates a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes and anaemia may be more dependent on the host immune response than on the direct destruction of red blood cells by the piroplasm (Boozer and Macintire 2003; Halder and Gupta, 2022) [2, 8].

Increase in level of alanine aminotransferase could be due to damage or abnormal function of biliary system. Increased activities of alanine aminotransferase were might be due to escape of these enzymes from the damaged hepatic parenchymal cells with necrosis or altered membrane permeability indicating hepatic dysfunction (Reddy *et al.*, 2016) [13]. 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 (Furlanello *et al.*, 2005; Gonmei *et al.*, 2020) [5, 7].

In this study, the main biochemical changes were hypoproteinaemia, hypoalbuminemia and increased serum levels of alanine aminotransferase, creatinine and blood urea nitrogen in majority of the infected dogs. This finding concurs with the previous reports (Furlanello *et al.*, 2005; Sai *et al.*, 2023) [5, 16].

**Table 1:** Table showing the clinical signs in dogs with *Babesia* infection.

| S. No | Clinical findings       | No. of dogs with Babesiosis (27) | Percentage of dogs (%) |
|-------|-------------------------|----------------------------------|------------------------|
| 1.    | Fever                   | 24                               | 88.88% (24/27)         |
| 2.    | Tick infestation        | 22                               | 81.48% (22/27)         |
| 3.    | Inappetence             | 22                               | 81.48% (22/27)         |
| 4.    | Depressed demeanor      | 21                               | 77.77% (21/27)         |
| 5.    | Pale mucus membranes    | 21                               | 77.77% (21/27)         |
| 6.    | Lymphadenopathy         | 19                               | 70.37% (19/27)         |
| 7.    | Emaciation              | 17                               | 62.96% (17/27)         |
| 8.    | Dyspnea                 | 14                               | 51.85% (14/27)         |
| 9.    | Tachycardia             | 14                               | 51.85% (14/27)         |
| 10.   | Tachypnoea              | 12                               | 44.44% (12/27)         |
| 11.   | Nervous signs           | 11                               | 40.74% (11/27)         |
| 12.   | Scleral congestion      | 10                               | 37.03% (10/27)         |
| 13.   | Icteric mucus membranes | 5                                | 18.52% (05/27)         |
| 14.   | Hemoglobinuria          | 3                                | 11.11% (03/27)         |
| 15.   | Dependent edema         | 2                                | 7.40% (02/27)          |
| 16.   | Hypothermia             | 2                                | 7.40% (02/27)          |

**Table 2:** Haematological findings in dogs with babesiosis (Mean± S.E)

| S. No | Parameters                                     | Apparently healthy dogs (10) | <i>Babesia</i> infected dogs (27) | Level of significance (p) |
|-------|------------------------------------------------|------------------------------|-----------------------------------|---------------------------|
| 1.    | Haemoglobin (g/dL)                             | 13.03 <sup>b</sup> ±0.42     | 9.52 <sup>a</sup> ±0.12           | 0.001 <sup>**</sup>       |
| 2.    | Total erythrocyte count (×10 <sup>6</sup> /μL) | 6.51 <sup>b</sup> ±0.19      | 4.73 <sup>a</sup> ±0.46           | 0.001 <sup>**</sup>       |
| 3.    | Packed cell volume (%)                         | 39.29 <sup>b</sup> ±2.14     | 28.20 <sup>a</sup> ±2.51          | 0.001 <sup>**</sup>       |
| 4.    | Total leucocyte (×10 <sup>3</sup> /μL)         | 9334.29 <sup>b</sup> ±81.34  | 8786.67 <sup>a</sup> ±71.45       | 0.047 <sup>*</sup>        |
| 5.    | Neutrophils (%)                                | 75.21 <sup>a</sup> ±1.2      | 82.91 <sup>b</sup> ±2.5           | 0.029 <sup>*</sup>        |
| 6.    | Lymphocytes (%)                                | 20.43 <sup>b</sup> ±0.85     | 13.03 <sup>a</sup> ±1.86          | 0.031 <sup>*</sup>        |
| 7.    | Eosinophils (%)                                | 3.81±0.27                    | 3.22±0.27                         | 0.056 <sup>NS</sup>       |
| 8.    | Monocytes (%)                                  | 2.09±0.27                    | 2.34±0.27                         | 0.061 <sup>NS</sup>       |
| 9.    | Platelet count (×10 <sup>3</sup> /μL)          | 2.71 <sup>b</sup> ±0.28      | 1.24 <sup>a</sup> ±0.21           | 0.03 <sup>*</sup>         |

\**p*<0.05 - Significant, \*\**p*<0.01 – highly significant and *p*>0.05 non-significant

**Table 3:** Serum biochemical findings in dogs with babesiosis (Mean±S.E)

| S.no | Parameters                  | Apparently healthy dogs (10) | <i>Babesia</i> infected dogs (27) | Level of significance (p) |
|------|-----------------------------|------------------------------|-----------------------------------|---------------------------|
| 1.   | Total Protein (gm/dL)       | 6.81 <sup>b</sup> ±0.22      | 4.48 <sup>a</sup> ±0.21           | 0.001 <sup>**</sup>       |
| 2.   | Albumin (gm/dL)             | 3.25 <sup>b</sup> ±0.04      | 1.23 <sup>a</sup> ±0.09           | 0.001 <sup>**</sup>       |
| 3.   | ALT (U/L)                   | 18.71 <sup>a</sup> ±1.56     | 53.00 <sup>b</sup> ±8.2           | 0.018 <sup>*</sup>        |
| 4.   | Alkaline Phosphatase (U/L)  | 87.05 <sup>a</sup> ±06.31    | 139.5 <sup>b</sup> ±14.83         | 0.002 <sup>**</sup>       |
| 5.   | Total bilirubin (mg/dL)     | 0.84 <sup>a</sup> ±0.05      | 1.12 <sup>b</sup> ±0.12           | 0.021 <sup>*</sup>        |
| 6.   | Direct bilirubin (mg/dL)    | 0.44 <sup>a</sup> ±0.02      | 0.74 <sup>b</sup> ±0.03           | 0.028 <sup>*</sup>        |
| 7.   | Indirect bilirubin (mg/dL)  | 0.40±0.03                    | 0.47±0.08                         | 0.31 <sup>NS</sup>        |
| 8.   | Blood urea nitrogen (mg/dL) | 16.56 <sup>a</sup> ±0.95     | 45.67 <sup>b</sup> ±2.55          | 0.041 <sup>*</sup>        |
| 9.   | Creatinine (mg/dL)          | 0.91 <sup>a</sup> ±0.07      | 1.57 <sup>b</sup> ±0.19           | 0.015 <sup>*</sup>        |

\**p*<0.05 - Significant, \*\**p*<0.01 – highly significant and *p*>0.05 non-significant

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