Haemato-biochemical Changes in Natural Cases of Canine Babesiosis

Showkat Ahmad Shah  
*Guru Angad Dev Veterinary and Animal Sciences University*

Naresh Kumar Sood  
*Guru Angad Dev Veterinary and Animal Sciences University*

Srinivasa Rao Tumati  
*Guru Angad Dev Veterinary and Animal Sciences University*

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Showkat Ahmad Shah, Naresh Kumar Sood and Srinivasa Rao Tumati
1Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana-141004, Punjab, India
2Department of Veterinary Pathology, SKAUST-K, Srinagar, India
3Department of Veterinary Public Health, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana-141004, Punjab, India

Corresponding Author: S.R. Tumati, Department of Veterinary Public Health, N.T.R. College of Veterinary Science, Gannavaram, Andhra Pradesh, India

ABSTRACT

Incidence of severe anemia due to babesiosis in dogs resulting in death in a short time of illness is on the increase in Punjab, during last few decades due to introduction of exotic breeds like Grey Hound, German shepherd, Doberman, Labrador and others. In the present study the hematological and biochemical changes in blood samples obtained from 4 dogs naturally infected with Babesia were evaluated. The dogs were presented to the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana from August 2008 to April 2009. The evaluation included Hemoglobin (Hb), Red Blood Cell count (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), leucocyte counts, platelet counts and reticulocyte counts. The serum biochemistry included Blood Urea Nitrogen (BUN), creatinine, total protein, albumin, bilirubin, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP). The hematological findings in most of these dogs were normocytic normochromic anemia and thrombocytopenia. The total and differential leukocyte counts were not specific. Biochemical values were within normal ranges but serum biochemistry varied for each dog. This study indicated that Babesia infection in dogs apparently caused anemia and thrombocytopenia, while other clinical values did not change.

Key words: Dog, Babesia, hemato-biochemical changes, anemia, thrombocytopenia

INTRODUCTION

Canine babesiosis caused by tick-borne organisms of the genus Babesia, is one of the most significant diseases worldwide. The commonly occurring Babesia species in dogs are the Babesia canis and Babesia gibsoni (Taboada and Merchant, 1991). The Sero-prevalence of babesiosis in the United States is higher in adult dogs than in dogs younger than 1 year but the lower prevalence was noted in kennels where more intensive tick controls were performed (Irwin, 2010). The immunological response plays the most important role in pathogenesis of canine babesiosis. Babesia initiates a mechanism of Antibody-mediated cytotoxic destruction of circulating erythrocytes. Autoantibodies are directed against components of the membranes of infected and uninfected erythrocytes. This causes intravascular and extravascular hemolysis which leads to
anemia and hemoglobinemia (Pedersen, 1999; Irwin, 2005). The typical clinical sign observed in animals with babesiosis is hemolytic anemia (Jacobson and Clark, 1994). Parasitemia results in increased osmotic fragility of erythrocytes (Makinde and Bobade, 1994) and serum hemolytic factors (Onishi and Suzuki, 1994). These factors induce hemolysis and subsequent anemia. Clinical signs of canine babesiosis include: Fever, anorexia, depression, oliguria, hemoglobinuria, vomiting, lethargy, dehydration, icterus, pale mucous membranes, splenomegaly and dyspnea (Irwin, 2005). Anemia and thrombocytopenia are the primary hematological abnormalities found in affected dogs (Furlanello et al., 2005). The most common anemia caused by large form of Babesia is normocytic and normochromatric. Both regenerative and non-regenerative anemias have been observed during the course of canine babesiosis (Furlanello et al., 2005). Considering leucocyte abnormalities most dogs show neutropenia and lymphopenia (Furlanello et al., 2005). There are no studies about hematological and biochemical changes in dogs naturally infected with Babesia in Ludhiana, Punjab, India. In the present study hematological and biochemical changes in 4 dogs naturally infected with large form of Babesia (3) and Babesia gibsoni (1) were investigated.

MATERIALS AND METHODS

Samples of blood were collected from dogs naturally infected with Babesia from August 2008 to April 2009. The diagnosis of babesiosis was confirmed by demonstration of the parasites within the infected erythrocytes in Wright-Giemsa stained thin blood smears (Matijatko et al., 2007). In these samples a complete blood count was performed with an automatic hematologic analyser (Beckman Coulter, Coulter diff Ac. T, USA). Ethylenediamine Tetraacetic Acid (EDTA) was used as an anticoagulant. The erythrocyte count, concentration of hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and thrombocyte count were evaluated. Red blood cell morphology and leukocyte count were performed with a microscope using peripheral blood smears stained with Giemsa. The blood samples were also collected in heparinised vials for biochemical analysis. Plasma was separated within 3 h from heparinised blood for the evaluation of the various blood chemistry parameters. Plasma concentration of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN), Total Protein (TP), albumin, creatinine and total bilirubin were determined by automated clinical chemistry analyzer (Vitros System Chemistry DT 60 11, Orthoclinical Diagnostics, Johnson and Johnson, USA) using standard kits (Vitros-Ortho-clinical Diagnostics, Mumbai). Reticulocyte count was done by counting reticulocytes in blood smears made from 0.5 mL of whole blood incubated with an equal volume of brilliant cresyl blue. Results were expressed as Means±Standard Deviation.

Statistical analysis: The arithmetical mean and standard deviation were calculated for all parameters using Windows, Ver 5.1 Release and Stat Soft.Inc.1984-1996.

RESULTS

Four cases of canine babesiosis including three cases of large forms of Babesia (B. canis) and one case of B. gibsoni were diagnosed during the period of study by microscopic examination of the stained blood smear (Fig. 1-4). There were four Babesia canis with in the red blood cell and these were of lightly basophilic pyriform structures with indistinct internal structures in Wright-Giemsa
B. canis inside the RBC

Extra erythrocytic from of B. canis

B. gibsoni inside the RBC
Fig. 4: Peripheral blood smear showing ghost cells (arrows), seen in hemolytic anemia as in babesiosis, Wright-Geimsa X 750

Ghost cells

stain (Fig. 1). Group of extra erythrocytic, free *Babesia* forms may result from rupture of the RBC with severe infection (Fig. 2). *B. gibsoni*, small pleomorphic form without pyriform shape was present in the centre of the blood smear (Fig. 3). Pale staining small red blood cells called ghost cells in the blood smear (Fig. 4) indicated intravascular hemolysis in hemolytic anemia caused by babesiosis. On clinical examination, most of the dogs were seen infested with ticks. The dogs mean rectal temperature was 103°F. The animals affected with babesiosis had normocytic, normochromic anemia and 50% of cases had a PCV less than 20%. The white blood cell counts in *Babesia* infected dogs ranged from 3.3-11.8×10^3 µL^-1 and majority (75%) had WBC counts lower than 6.0×10^3 µL^-1. In this study, leukocyte abnormalities were non-specific as either normal leukocyte count or leukopenia was observed. All the *Babesia* infected dogs had thrombocytopenia. The serum chemistry includes BUN, creatinine, total protein, albumin, bilirubin, ALT, AST remained unaltered in canine babesiosis, except for an increase in ALP. The mean hematological and serum biochemical values of uncomplicated cases of canine babesiosis are presented in Table 1.

DISCUSSION
The hematological findings of canine babesiosis in the present study were in agreement with the findings of Furlanello et al. (2005) in infected dogs with *Babesia* in Northern Italy, however, microcytic hypochromic anemia and thrombocytopenia were common clinical findings in *Babesia* infected dogs in Bangkok (Niwetpathomwat et al., 2006). It is speculated that the anemia in babesiosis resulted from an increased osmotic fragility of erythrocytes and thrombocytopenia due to immune-mediated platelets destruction (Makinde and Bobade, 1994). Increased erythropagocytic activity of macrophages and immune-mediated cleavage are the significant pathogenetic mechanisms of anemia in babesiosis (Onishi and Suzuki, 1994). Additionally oxidative stress in babesiosis may cause damage to erythrocytes that result in their increased susceptibility to phagocytosis (Murase et al., 1996; Tvedten, 2004). The decrease of hemoglobin concentration below the reference value in 100% cases in the present investigation resulted from extravascular hemolysis (Breckus and Andreasen, 2003). The most common abnormality in the investigated parameters of babesiosis was thrombocytopenia. The reason for thrombocytopenia in babesiosis
Table 1: Hematological and biochemical findings (Mean±SE) in canine babesiosis (n = 4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Mean±SE</th>
<th>Observation range</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>g.dL⁻¹</td>
<td>6.65±0.86</td>
<td>4.40-9.0</td>
<td>12.0-18</td>
</tr>
<tr>
<td>RBC</td>
<td>x10⁶ µL⁻¹</td>
<td>3.54±0.37</td>
<td>2.90-4.5</td>
<td>5.5-8.8</td>
</tr>
<tr>
<td>PCV</td>
<td>%</td>
<td>22.10±0.64</td>
<td>13.40-30.0</td>
<td>37.0-55</td>
</tr>
<tr>
<td>MCV</td>
<td>Fl</td>
<td>60.87±4.31</td>
<td>48.00-70.0</td>
<td>60.0-77</td>
</tr>
<tr>
<td>MCH</td>
<td>Pg</td>
<td>18.67±0.91</td>
<td>16.10-20.6</td>
<td>19.5-24.5</td>
</tr>
<tr>
<td>MCHC</td>
<td>%</td>
<td>31.05±1.86</td>
<td>25.71-35.3</td>
<td>32.0-36.0</td>
</tr>
<tr>
<td>WBC</td>
<td>x10³ µL⁻¹</td>
<td>6.37±1.68</td>
<td>3.30-11.8</td>
<td>6.0-17</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>%</td>
<td>67.25±16.12</td>
<td>10.00-89.0</td>
<td>60.0-70</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>%</td>
<td>12.00±0.89</td>
<td>6.00-12.0</td>
<td>30.0-40.0</td>
</tr>
<tr>
<td>Platelets</td>
<td>x10³ µL⁻¹</td>
<td>100.0±18.25</td>
<td>50.00-150.0</td>
<td>200.0-500.0</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>%</td>
<td>2.00±0.33</td>
<td>1.20-3.0</td>
<td>0.0-1.5</td>
</tr>
<tr>
<td>BUN</td>
<td>mg.dL⁻¹</td>
<td>15.60±1.96</td>
<td>12.00-22.0</td>
<td>7.0-32</td>
</tr>
<tr>
<td>Creatinine</td>
<td>mg.dL⁻¹</td>
<td>0.82±0.11</td>
<td>0.50-1.1</td>
<td>0.5-1.4</td>
</tr>
<tr>
<td>Total protein</td>
<td>g.dL⁻¹</td>
<td>5.65±0.32</td>
<td>4.00-6.5</td>
<td>5.3-7.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>g.dL⁻¹</td>
<td>3.17±0.18</td>
<td>2.60-3.5</td>
<td>3.2-4.2</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>mg.dL⁻¹</td>
<td>1.25±0.13</td>
<td>0.50-1.5</td>
<td>0.2-1.3</td>
</tr>
<tr>
<td>ALP</td>
<td>IU.L⁻¹</td>
<td>139±26.14</td>
<td>125.00-152.0</td>
<td>0.0-90.0</td>
</tr>
<tr>
<td>ALT</td>
<td>IU.L⁻¹</td>
<td>35.50±11.81</td>
<td>20.00-75.0</td>
<td>10.0-94.0</td>
</tr>
<tr>
<td>AST</td>
<td>IU.L⁻¹</td>
<td>37.50±1.68</td>
<td>35.00-43.0</td>
<td>10.0-62</td>
</tr>
</tbody>
</table>


may be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation (Boozer and Macintire, 2003). In this study elevated body temperature was recorded and this elevated body temperature could have contributory effect on thrombocytopenia (Oglesbee et al., 1999). Lymphocytopenia was found in all cases in this study and this observation is contrary with the findings of Latimer and Prasse (2003). Lymphocytopenia may be due to concurrent viral infection associated with babesiosis.

CONCLUSION

The hematology and blood chemistry values in this study are similar to the results observed in other reports. The most significant abnormalities that are usually found in canine babesiosis are normocytic normochromic anemia and thrombocytopenia.

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