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Haemato-biochemical Changes in Natural Cases of Canine Babesiosis

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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 dispnea (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 normochromatic. 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 amino Transferase (AST), Alanine amino Transferase (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

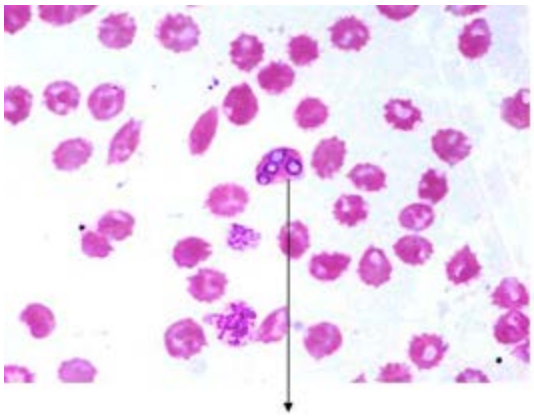

Fig. 1: Peripheral blood smear showing an erythrocyte with large form of *Babesia* (*B. canis*, arrow), Wright-Geimsa X 1000

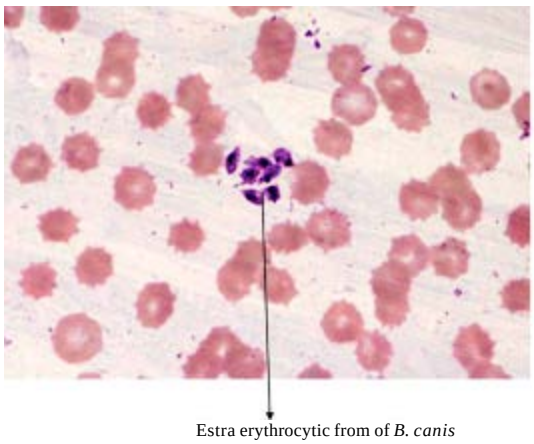

Fig. 2: Peripheral blood smear revealing extra erythrocytic forms of large *Babesia* (*B. canis*, arrow), Wright-Geimsa X 1000

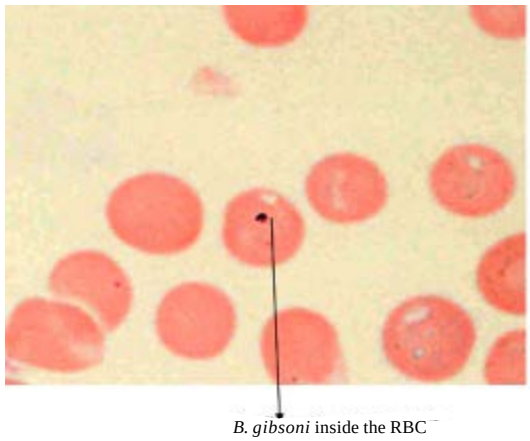

Fig. 3: Peripheral blood smear revealing an erythrocyte infected with *Babesia gibsoni* (arrow), Wright-Geimsa X 1500

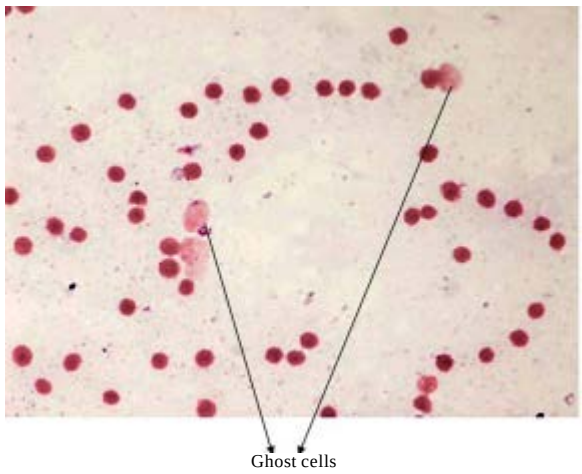

Fig. 4: Peripheral blood smear showing ghost cells (arrows), seen in hemolytic anemia as in babesiosis, Wright-Geimsa X 750

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 vin 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 erythrophagocytic 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 (Brockus 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)

		Patient data		
Parameter	Units	Mean±SE	Observation range	Reference range
Hb	g dL^{-1}	6.65±00.86	4.40-9.0	12.0-18
RBC	$\times 10^6$ μL^{-1}	3.54±00.37	2.90-4.5	5.5-8.8
PCV	%	22.10±03.64	13.40-30.0	37.0-55
MCV	Fl	60.87±04.31	48.30-70	60.0-77
MCH	Pg	18.67±00.91	16.10-20.6	19.5-24.5
MCHC	%	31.05±01.86	25.71-35.3	32.0-36
WBC	$\times 10^3$ μL^{-1}	6.37±01.68	3.30-11.8	6.0-17
Neutrophils	%	67.25±16.12	10.00-89	60.0-70
Lymphocytes	%	12.00±00.89	6.00-12	30.0-40
Platelets	$\times 10^3$ μL^{-1}	100.00±18.25	50.00-150	200.0-500
Reticulocytes	%	2.00±00.33	1.20-3.0	0.0-1.5
BUN	mg dL^{-1}	15.60±01.96	12.00-22.0	7.0-32
Creatinine	mg dL^{-1}	0.82±00.11	0.50-1.1	0.5-1.4
Total protein	g dL^{-1}	5.65±00.32	4.90-6.5	5.3-7.6
Albumin	g dL^{-1}	3.17±00.18	2.60-3.5	3.2-4.2
Bilirubin	mg dL^{-1}	1.25±00.13	0.90-1.5	0.2-1.3
ALP	IU L^{-1}	139.2±06.14	125.00-152.0	0.0-90
ALT	IU L^{-1}	35.50±11.81	20.00-75.0	10.0-94
AST	IU L^{-1}	37.50±01.69	35.00-43.0	10.0-62

Hb: Haemoglobin, RBC: Red blood cell count, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean haemoglobin concentration, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cell count, BUN: Blood urea nitrogen, ALP: Alkaline phosphatase, ALT: Alanine amino transferase, AST: Aspartate amino transferase

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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REFERENCES

Boozer, A.L. and D.K. Macintire, 2003. Canine babesiosis. Vet. Clin. North Am. Small Anim. Pract., 33: 885-904.

Brockus, C.W. and C.B. Andreasen, 2003. Erythrocytes. In: Clinical Pathology, Latimer, K.S., E.A. Mahaffey and K.W. Prasse (Eds.). 4th Edn., Blackwell Publishing Company, Ames., pp: 3-45.

Furlanello, T., F. Fiorio, M. Caldin, G. Lubas and L. Solano-Gallego, 2005. Clinicopathological findings in naturally occurring cases of babesiosis caused by large form *Babesia* from dogs of Northern Italy. Vet. Parasitol., 134: 77-85.

Irwin, P., 2005. Babesiosis and Cytauxzoonosis. In: Arthropod-borne Infectious Diseases of the Dog and Cat, Shaw, S.E. and M.J. Day (Eds.), Manson Publishing, Barcelona, pp: 63-77.

Irwin, P.J., 2010. Canine babesiosis. Vet. Clin. North Am. Small Anim. Practice, 40: 1141-1156.

Jacobson, L.S. and I.A. Clark, 1994. The pathophysiology of canine babesiosis: New approaches to an old puzzle. J. S. Afr. Vet. Assoc., 65: 134-145.

Latimer, K.S. and K.W. Prasse, 2003. Leukocytes. In: Clinical Pathology, Latimer, K.S., E.A. Mahaffey and K.W. Prasse (Eds.). 4th Edn., Blackwell Publishing Company, Ames., pp: 46-79.

Makinde, M.O. and P.A. Bobade, 1994. Osmotic fragility of erythrocytes in clinically normal dogs and dogs with parasites. Res. Vet. Sci., 57: 343-348.

Matijatko, V., V. Mrljak, I. Kis, N. Kucer and J. Forsek *et al*., 2007. Evidence of an acute phase response in dogs naturally infected with *Babesia canis*. Vet. Parasitol., 144: 242-250.

Murase, T., T. Ueda, O. Yamato, M. Tajima and Y. Maede, 1996. Oxidative damage and enhanced erythrophagocytosis in canine erythrocytes infected with *Babesia gibsoni*. J. Vet. Med. Sci., 58: 259-261.

Niwetpathomwat, A., S. Techangamsuwan, S. Suvarnavibhaja and S. Assarasakorn, 2006. A retrospective study of clinical hematology and biochemistry of canine babesiosis on hospital populations in Bangkok, Thailand. Comp. Clin. Pathol., 15: 110-112.

Oglesbee, M.J., K. Diehl, E. Crawford, R. Kearns and S. Krakowka, 1999. Whole body hyperthermia: Effects upon canine immune and hemostatic functions. Vet. Immunol. Immunopathol., 69: 185-199.

Onishi, T. and S. Suzuki, 1994. Changes of serum hemolytic activity and the number of reticulocytes in canine *Babesia gibsoni* infection. J. Vet. Med. Sci., 56: 611-612.

Pedersen, N.C., 1999. A review of immunologic diseases of the dog. Vet. Immunol. Immunopathol., 69: 251-342.

Taboada, J. and S.R. Merchant, 1991. Babesiosis of companion animals and man. Vet. Clin. North Am. Small Anim. Pract., 21: 103-123.

Tvedten, H., 2004. Reference Values. In: Small Animal Clinical Diagnosis by Laboratory Methods, Willard, M.D. and H. Tvedten (Eds.) 4th Edn., Elsevier, Missouri, USA., pp: 417-419.