International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; SP4: 25-28

Praveen Kumar
Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science and AH, Kanke Ranchi, Jharkhand, India

Abhishek Kumar
Department of Veterinary Medicine, College of Veterinary Science and AH, Kanke Ranchi, Jharkhand, India

Correspondence
Praveen Kumar
Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science and AH, Kanke Ranchi, Jharkhand, India

(Special Issue -4)
International Conference on Food Security and Sustainable Agriculture
(Thailand on 21-24 December, 2018)

Haemato-biochemical changes in dogs infected with *Babesiosis*

Praveen Kumar and Abhishek Kumar

Abstract
Canine babesiosis is an important and potentially life-threatening disease in India, caused by *B. canis or B. gibsoni*. In the present study the hemato-biochemical changes were observed in canine babesiosis. Twenty dogs positive for babesiosis, were selected for the study. Diagnosis was made on the basis of clinical signs and demonstration of *Babesia* organism in Giemsa-stained blood smears. The clinical signs were recorded and blood samples were subjected to estimation of hemato-biochemical parameters. The clinical cases were manifested by wide variety of non-specific clinical signs. The haematological evaluation revealed that the mean values of haemoglobin and total erythrocyte counts in dogs with babesiosis decreased significantly in comparison to healthy dogs. Among differential leukocyte count, mean values of neutrophils and eosinophils increased while lymphocytes decreased in dogs with babesiosis in comparison to healthy dogs. Serum biochemistry revealed increase value of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase as well as decrease in albumin and glucose levels in dogs with babesiosis as compared to healthy dogs.

Keywords: babesiosis, haemato-biochemical changes, dogs

Introduction
Blood-feeding ectoparasites such as ticks, fleas, sand flies, and mosquitoes can transmit many dangerous pathogens to dogs. Tick-borne hemoparasites are one of the most important vector-borne infections of dogs. Tick-borne hemopathogens such as Babesia, Ehrlichia, Anaplasma, etc are of major health concern to dogs. Canine babesiosis is a clinically significant and geographically widespread hemoprotozoan disease of domesticated dogs (Taboada and Merchant; 1991) [22]. The commonly occurring *Babesia* species in dogs are *Babesia canis* and *Babesia gibsoni* (Lobetti; 2004) [9]. The diagnosis is made by demonstrating *Babesia* organisms within infected erythrocytes on a blood smear stained with a Romanowski-type stain (Shrivastava *et al.* 2013) [18]. The disease appears during the whole year period, with frequent outbreaks in the spring and autumn. Canine babesiosis can vary from peracute and fatal to chronic and subclinical, depending on microbial virulence and host resistency (Torbica *et al.* 2013) [23]. Large pear shaped organisms usually present in pairs are indicative of *B. canis* infection, whereas smaller, singular, round to oval organisms are *B. gibsoni* (Lobetti; 2004) [9]. Ectoparasites such as ticks, fleas, sand flies can transmit many dangerous pathogens to dogs. The parasite also transmitted by blood exchange and transplacental route can also be possible (Jefferies; 2007) [7]. A wide range of clinical signs have been reported in canine babesiosis. The typical clinical findings include anemia, thrombocytopenia, leukocyte abnormalities, increased liver enzymes, and Hyperbilirubinemia. Hypokalemia, hyperglobulinemia, azotemia, metabolic acidosis, and abnormalities of urinalysis may be observed in some severely affected dogs (Irwin and Hutchinson; 1991). The disease can be clinically classified into uncomplicated and complicated forms. For some time, uncomplicated babesiosis has been suggested to be a consequence of haemolysis (Jacobson and Clark 1994) [6] while complicated canine babesiosis has been suggested to be a consequence of the development of systemic inflammatory

response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), both of which are cytokine-mediated phenomena (Jacobson and Clark 1994; Welzl *et al.*, 2001) [6, 26]. Both uncomplicated and complicated babesiosis appear to be the result of host inflammatory responses (Matijatko *et al.*, 2007; Schetters *et al.*, 2009) [11]. The immunological response plays the most important role in pathogenesis of canine babesiosis. These parasites initiate a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes. Autoantibodies are directed against components of the membranes of infected and uninfected erythrocytes which causes intravascular and extravascular hemolysis. The clinical-pathological changes, including hematology and blood chemistry, are nonspecific. The various clinical symptoms regularly depend on the severity of the disease in infected animals. Present study was conducted to find out the hemato-biochemical changes in cases of canine babesiosis.

Materials and Methods

This work was conducted in dogs brought to Veterinary Clinical Complex Ranchi, which were examined clinically from March 2016 to Feb 2017, irrespective of their age, breed and sex. Peripheral blood smears were made from suspected dogs and examined for the presence of intra-erythrocytic piroplasms. Apparently healthy dogs free from ectoparasites, reported to clinics for check-up, were used as healthy control. Blood was collected from them aseptically. Peripheral blood smears were made for diagnosis of babesiosis and was confirmed by cytological examination by standard procedure. Twenty dogs positive for babesiosis, based on the presence of *Babesia* in blood smears and six apparently healthy dogs were selected for the study. Approximately 6 ml of blood samples were collected from positive dogs in a sterile anticoagulant vial containing ethylenediaminetetraacetic acid (EDTA) and another 6ml of blood were also collected for serum on day 0 through cephalic or recurrent tarsalvein puncture. Haematological parameters including total erythrocyte count (TEC, millions/u1), haemoglobin (Hb, g/ dl), packed cell volume (PCV, %), total leukocyte count (TLC), differential leukocyte count (DLC, %) and platelet (thousands/u1) were estimated following standard procedure. Biochemical parameters including alanine transaminase (ALT, U/L), aspartate transaminase (AST, U/L), alkaline phosphatase (ALP, U/L), creatine kinase (CK, U/L), bilirubin (mg/dl), total protein (g/dl) and albumin (g/dl), blood urea nitrogen (mg/dl), glucose (mg/dl) and creatinine (mg/dl), were determined using Blood Chemistry Semi Auto Analyzer using standard kits. Hemato-biochemical changes in dogs with babesiosis were compared with healthy dogs. The data obtained were subjected to the statistical analysis described by Snedecor and Cochran (1990) [20].

Results and Discussion

Rise in temperature was observed in 16 (80%) cases while remaining 4 (20%) of the dogs had slightly elevated temperature. These findings were in agreement with Torbica *et al.* 2013 [23]. The respiration rate and heart rate were increased than healthy dogs (Table 1) and this may be due to anaemia and stress Matijatko *et al.* 2014 [12]. The tick infestation was found in 19 (95%) cases. Godara *et al.* (2010) [3] stated that there was a positive correlation between the presence of ticks on the body surface of host and hemoprotozoan infections. Haemoglobinuria was rarely observed in this study. In the affected dogs, total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume (PCV) decreased significantly indicating anaemia in the affected dogs (Table 2). These findings were in accordance with Maele *et al.* 2008 [10]. Anaemia results from the hemolysis due to multiplication of the organism in peripheral vessels, direct mechanical disruption caused by parasite as it leaves in red blood cells, intravascular hemolysis, and immune-mediated or non-immune mediated destruction of red blood cells. The TLC was increased significantly in the affected animals with significant increase in lymphocyte percent and monocyte percent. Selvaraj *et al.* (2010) [16] reported an increased leukocyte count with significant increase in neutrophils. There was Significant thrombocytopenia in the affected animals. Similar findings were reported by Fabisiak *et al.* (2010) [2]. Sivajothi *et al.* (2014) [14] who reported significant decreased platelet counts i.e., thrombocytopenia. The mechanisms of the thrombocytopenia are not yet fully understood in babesiosis. Elevated body temperature could have contributory effect on thrombocytopenia (Oglesbee *et al.* 1999) [13]. Thrombocytopenia observed in canine babesiosis might be due to immune mediated destruction of thrombocytes or splenic sequestration or coagulatory consumption of platelets from haemolytic or vascular injury (Solano - Gallego and Baneth 2011) [11].

Table 1: Physical parameters in healthy and babesiosis positive dogs.

Clinical variant	Healthy dogs	Babesiosis Positive
Rectal temperature (°F)	102.60±00.21	104.80±00.23**
Heart rate (beats/min)	114.43±02.64	133.73±02.89**
Respiration rate (breaths/min)	29.00±01.98	38.00±02.56**

Table 2: Comparison of hematological parameters in healthy and babesiosis positive dogs.

Parameter	Healthy dogs	*Babesiosis* positive
Hb (g/dl)	13.23±00.43	09.33±00.80**
TEC (×106/µl)	05.89±00.86	02.98±00.39**
PCV (%)	39.86±00.64	21.64±00.72*
TLC (×103/µl)	16.39±01.01	31.14±01.87**
Neutrophils (%)	67.59±01.13	79.71±01.94**
Lymphocytes (%)	25.07±00.79	14.17±01.24**
Monocytes (%)	02.56±00.59	03.06±00.29
Eosinophils (%)	02.19±00.29	04.67±00.45**
Basophils (%)	00.00±00.00	00.00±00.00
Platelet count (×103/µl)	297.67±14.21	167.66±11.99**

Table 3: Comparison of serum biochemical parameters (mean±SE) in healthy and babesiosis positive dogs.

Parameter	Healthy dogs	Babesiosis positive dogs
ALP (IU/L)	91.71±03.63	274.61±24.57**
ALT (IU/L)	48.53±02.69	97.96±04.42**
AST (IU/L)	57.33±02.19	79.33±02.72**
Total bilirubin (mg/dl)	00.51±00.09	01.27±00.19**
BUN (mg/dl)	16.67±01.08	6.82±00.94**
Creatinine (mg/dl)	01.13±00.16	02.39±00.19**
Glucose (mg/dl)	87.43±00.72	58.91±00.63**
Total protein (g/dl)	05.87±00.22	05.27±00.52
Albumin (g/dl)	02.97±00.19	01.22±00.09**

The levels of ALT, AST, ALP and bilirubin were significantly higher in the infected dogs (Table 3). These changes may be due to the haemolysis and cellular damage to the hepatic cells. These findings were in accordance with Shah *et al.* (2011) [17] and Wadhwa *et al.* (2011) [25]. Increase in level of ALP was may be due to damage or abnormal

function of biliary system (Crnogaj *et al.*; 2010). Increased activities of AST and 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 (Gupta *et al.*; 2002) [4]. Hyperbilirubinemia was due to resulted from both intravascular and extra-vascular haemolysis (Irwin and Hutchinson; 1991) [5].

There was a significant decrease in the values of BUN in the affected animals. Lower values might be observed due to protein malnutrition or hepatic insufficiency. In the present study, significant decrease in the blood glucose level was observed. Similar findings were reported by Matijatko *et al.* (2007) [11]. The lower levels may be due to starvation and hepatic dysfunction. Hypoglycaemia could be as a result of sepsis causing anorexia, impaired hepatic function and increase in breakdown in system (Konto *et al*., 2014) [8].

The level of serum creatinine was significantly higher in the infected dogs. Reddy *et al.* (2014) [14] reported significant increase in creatinine in babesiosis positive dogs. Levels of albumin decreased significantly in dogs with babesiosis than healthy dogs. These results were in accordance with Yadav *et al.* (2011) [27] and Vijayalakshmi *et al*. (2014) [24] who observed same findings in canine babesiosis. This might be due to involvement of liver or kidney. The difference between values of total protein in babesiosis positive dogs and healthy dogs were statistically non-significant. These findings were in accordance with Reddy *et al.* (2014) [14].

Conclusion

All the suspected dogs in this study were diagnosed only based on the microscopic examination of the stained peripheral blood smears. The dogs infected with babesiosis exhibited clinical signs include tick infestation, emaciation, anorexia, Weakness, emaciation, dehydration, recumbancy, pale mucus membrane, congested, anorexia, dullness/depression, weak pulse and dullness. Hematologically there was decreased level of Hb, TEC, and PCV as well as increased level of TLC, neutrophils while serum biochemical pattern include increased level of ALP, ALT, AST and bilirubin while there is decrease in glucose.

References

1. Crnogaj M, Petlevski R, Mrljak V, Kis I, Torti M, Kucer N. *et al*. Malondialdehyde levels in serum of dogs infected with *Babesia canis*. Vet. Med. 2010; 55(4):163-171.
2. Fabisiak M, Sapicrzynski R, Klucinski W. Analysis Of hematological abnormalities obscrvcd in dogs infected by a large Babesia. Bull Vet Inst Pulawy. 2010; 54:167-170.
3. Godara R, Sharma RL, Sharma CS, Sharma DK. Parasitic infections in dogs in semi-arid Jaipur (Rajasthan). J Vet. Parasitol. 2010; 24:83-86.
4. Gupta SK, Bhardwaj RK, Singh R, Raina AK, Avinash K. Management of canine babesiosis - A case report. Indian J Vet. Med. 2002; 22:124.
5. Irwin PJ, Hutchinson GW. Clinical and pathological findings of *Babesia* infection in dogs. Aust. Vet. J. 1991; 68:204-209.
6. Jacobson L, Clark SIA. The pathophysiology of canine babesiosis: new approaches to an old puzzle. JS Afr. Vet. Assoc. 1994; 65:134-145.
7. Jefferies R, Ryan UM, Jardine J, Broughton DK, Robertson ID, Irwin PJ. Blood, bull terriers and babesiosis. Further evidence for direct transmission of *Babesia gibsoni* in dogs. Aust. Vet. J. 2007; 85:459-460.
8. Konto M, Biu AA, Ahmed MI, Mbaya AW, Luka J. Clinico-biochemical responses of dogs to experimental infection with *Babesia canis*. Veterinary World. 2014; 7(3):113-118.
9. Lobetti R. Hematological changes associated with tick-borne diseases. In: 29th World Cong, Greece, 6-9 Oct. WSAVA, 2004.
10. Maele IV, Bataille KS, Gielen I, Darninet S. An unusual form of canine babesiosis. Can. Vet. J. 2008; 49:283-286.
11. Matijatko V, Mrljak V, Kis I, Kučer N, Foršek J, Živičnjak T. Evidence of acute phase response in dogs naturally infected with *Babesia canis*. Vet. Parasitol. 2007; 144:242-250.
12. Matijatko V, Torti M, Kis I, Smit I, Stokovic I, Vranjesđuric S. *et al.* Serum cortisol and insulin concentrations in dogs naturally infected with *Babesia canis*. Vet. Arhiv. 2014; 84:551-562.
13. Oglesbee MJ, Diehl K, Crawford E, Kearns R, Krakowka S. Whole body hyperthermia: Effects upon canine immune and hemostatic functions. Vet. Immunol. Immunopathol. 1999; 69:185-199.
14. Reddy BS, Sivajothi S, Reddy LSS, Raju KGS. Clinical and laboratory findings of *Babesia* infection in dogs. J Parasit. Dis. 2014; 92:268-272.
15. Schetters TPM, Kleuskens JAGM, Van De Crommert J, De Leeuw, PWJ, Finzio A.-L, Gorenflot A. Systemic inflammatory responses in dogs experimentally infected with *Babesia canis*: a haematological study. Vet. Parasitol. 2009; 162:7-15.
16. Selvaraj P, Kumar KS, Vairamuthu S, Prathaban S, Srinivasan SR. *Babesia gibsoni* - An emerging challenge in canine pediatric practice in Chennai. Tamilnadu. J Vet. Anim. Sci. 2010; 6(3):122-124.
17. Shah SA, Sood N, Tumati SR. Haemato-biochemical changes in natural cases of canine babesiosis. Asian J Anim. Sci. 2011; 5(6):387-392.
18. Shrivastava S, Shukla PC, Sway M. Hemato-Biochemical Alterations in Naturally Occurring Cases of Canine Babesiosis. Environment & Ecology. 2013; 31(1):342-343.
19. Sivajothi S, Reddy BS, Rayulu VC, Venkatasiva kumar R. Babesiosis in dogs: A report of two different cases. Adv. Appl. Sci. Res. 2014; 5(3):276-279.
20. Snedecor GW, Cochran WG. Statistical Methods. 6th ed. Oxford and JBH Publishing, New York, 1990.
21. Solano-Gallego L, Baneth G. Babesiosis in dogs and cats- Expanding parasitological and clinical spectra. J Vet. Parasitol. 2011; 181:48-60.
22. Taboada J, Merchant SR. Babesiosis of companion animals and man. Vet. Clin. North Am. Small Anim. Pract. 1991; 21:103-123.
23. Torbica G, Bedrica L, Samardzija M, Lipar M, Ljubojevic D, Kreszinger M. *et al.* Canine babesiosis treatment with three different medicines Acta Veterinaria (Beograd). 2013; 63(2-3):279-290.
24. Vijayalakshmi P, Srinivasan SR, Vairamuthu S, Mangalagowri A, Latha BR, Nambi AP. Clinico Pathological Features in Dogs Associated with Babesiosis. Ind. Vet. J. 2014; 91(04):21-24.
25. Wadhwa DR, Pal B, Mandial RK, Kumar A, Agnihotri RK. Clinical, haemato biochemical and therapeutic studies of canine Babesiosis in Kangra valley of Himachal Pradesh. J Vet. Parasitol. 2011; 25(1):39-41.

26. Welzl C, Leisewitz AL, Jacobson LS, Vaughan-Scott T, Myburgh E. Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. JS. Afr. Vet. Assoc. 2001; 72:158-162.
27. Yadav R, Gattani A, Gupta SR, Sharma CS. Jaundice in dog associated with babesiosis - A case report. Int. J Agro Vet. Med. Sci. 2011; 5(1):3-6.