CLINICO-HAEMATO-BIOCHEMICAL CHANGES IN NATURALLY OCCURRING CANINE BABESIOSIS IN PUNJAB, INDIA

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ABSTRACT. A comprehensive study was conducted on the clinical observations including clinical history, physical examination along with haemato-biochemical alteration on 41 naturally occurring cases of canine babesiosis from Punjab state, India. Examination of 964 dogs revealed 4.25 percent (41/964) prevalence of the disease including 3.84 percent (37) *B. gibsoni* and 0.41 percent (4) *B. canis* infected cases. Clinical and parasitological diagnosis was finally confirmed by polymerase chain reaction. A large variation of clinical manifestations including rare findings of paraplegia, blindness, ocular bleeding, immune mediated haemolytic anaemia (IMHA), ascites and skin lesions were observed among the affected animals. Blood films showed anisocytosis and nucleated erythrocytes indicating regenerative anaemia. Blood parameters of the affected dogs revealed significant decrease in Hb, TEC, PCV and thrombocytes. Significant decrease in lymphocytes was found in *B. gibsoni* affected animals. The affected dogs showed significant increase in serum bilirubin, ALT, AKP, BUN and creatinine. Haemato-biochemical observations were indicative of severity of babesiosis in dogs

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

INTRODUCTION

Babesiosis is one of the most important tick-transmitted apicomplexan haemoprotozoan diseases that infect a wide range of vertebrate hosts and cause severe diseases in wild and domesticated animals (Kultler and Ristic, 1988). In dogs, babesiosis is reported worldwide (Ishibashi *et al*., 1995; Dev *et al*., 2004; Garcia, 2006) including various parts of India (Harikrishnan *et al*., 2005; Kumar *et al*., 2006) and Punjab state (Kalra and Singh, 1984; Eljadar 2010; Eljadar *et al*., 2012; Singla *et al*., 2014; Gonde *et al.,* 2016). This life-threatening disease is caused by various species of intraerythrocytic protozoan parasites of genus Babesia. The variable prevalence of both *B. canis* and *B. gibsoni* has been observed in India (0.66 to 21.7%). In Punjab state, the higher prevalence was

recorded (15.04%) for a small form (*B. gibsoni)* as compared to (0.26%) large form (*B. vogeli*) of *Babesia* spp. (Singla *et al.,* 2016). The pathogenesis of canine babesiosis varies in different regions (Saud and Hazarika, 2000), possibly owing to species variations of *Babesia* and various ecological conditions (Purnell, 1981). Clinically, cases of canine babesiosis are presented with a wide variation of signs, ranging from a hyperacute shock associated haemolytic crisis to an inapparent, sub-clinical infection (Gonde *et al.,* 2014). The severity of babesiosis is related to the extent of parasite replication in the host's red blood cells with subsequent cell lysis and a wide variation of clinical signs like anorexia, lethargy, haemolytic anaemia, icterus, vomiting and marked loss of body condition have been observed (Ettinger and Feldman, 2005; Vial and Gorenflot, 2006) along with variable clinico-pathological abnormalities including haemoglobinuria, hypoglycaemia, acid-base disturbances, azotaemia and elevations in the levels of liver enzymes (Irwin, 2010). *Babesia canis* and *B. gibsoni* are recognised as the two species responsible for causing canine babesiosis worldwide. *Babesia canis* usually occurs as a single pear-shaped piroplasm or in pairs of merozoites. *Babesia gibsoni* is a small parasite that commonly appears as individual ring forms or pyriform bodies. Both organisms have ixodid tick vectors and are found throughout Asia, Africa, Europe, the Middle East and North America. Birkenheuer *et al.* (1999) diagnosed *B. gibsoni* in puppies as young as 10 days of age possible due to transplacental transmission (Fukumoto *et al*., 2005), a time interval that is shorter than the prepatent period following tick transmission. To diagnose clinical cases the most reliable and practiced technique is Giemsa stained thin blood smear examination. This technique has got low sensitivity which renders it unsuitable for diagnosis of the subclinical and chronic form of the disease. Haemolytic anaemia, thrombocytopenia, anisocytosis and neutrophilia are the haemotological changes recorded by various workers from time to time (Conrad *et al*., 1991; Suh and Chung, 2000; Suarez *et al*., 2001; Lobetti, 2006). As there is no detailed study on clinico-haemato-biochemical changes in naturally occurring canine babesiosis from Punjab state India, hence in this communication we are reporting a comprehensive study on the clinical observations including clinical history, physical examination alongwith haemato-biochemical alteration in 41 naturally occurring cases of canine babesiosis from Punjab State, India.

MATERIALS AND METHODS

A total of 41 dogs aged between 2 months to 10 years positive for babesiosis were studied at Small Animal Clinics, Teaching Veterinary Hospital, GADVASU, Ludhiana, Punjab. After a comprehensive history, detailed clinical manifestations were noted and clinical parameters were recorded. Samples of blood were collected from the cephalic vein of dogs naturally

infected with *Babesia,* from January 2013 to December 2013. Diagnosis was made on the basis of clinical signs and demonstration of *Babesia* organism in Giemsa stained thin blood smears (Figure 1a and b) and confirmed by PCR (Figure 2). Blood was also collected from 10 healthy dogs for establishing control values. In these samples a complete blood count was performed with an automatic hematologic analyser (Beckman Coulter, Coulter diff Ac. T, USA). Ethylenediamine Tetra-acetic Acid (EDTA) at 1.5 mg/ml of blood, was used as an anticoagulant. The erythrocyte count, concentration of haemoglobin, haematocrit and thrombocyte count were evaluated. Leukocyte counts were performed with a microscope using peripheral blood smears stained with Giemsa. The blood samples collected in sodium fluoride vials were used for blood glucose estimation. For estimation of biochemical parameters 2 ml of blood was collected without anticoagulant in sterile syringes. Serum concentration of alanine amino transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), total protein (TP), blood glucose, albumin, globulin, creatinine and total bilirubin were determined by automated clinical chemistry analyser (Vitros System Chemistry DT 60 11, Orthoclinical Diagnostics, Johnson and Johnson, USA) using standard kits (Vitros-Ortho-clinical Diagnostics, Mumbai). Results were expressed as means ± standard deviation.

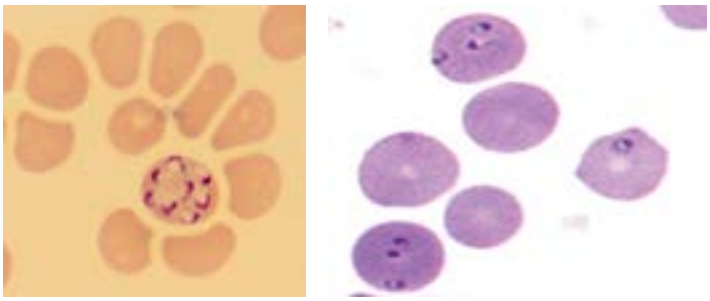

Figure 1. Giemsa stained blood smear of the infected dogs with pear shaped *B. canis* (a) and small circular shaped trophozoites of *B. gibsoni* (b) in erythrocytes.

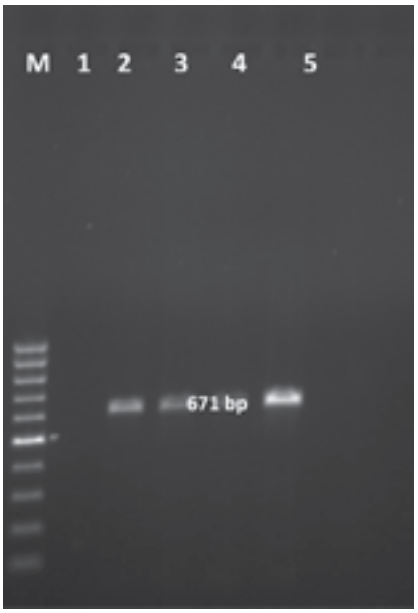

Figure 2. Gel electrophoresis showing *B. gibsoni* PCR assay. Lane M: GeneRuler 100 bp Ladder, lanes 2–4 field collected samples, lane 1: negative control and lane 5: positive control.

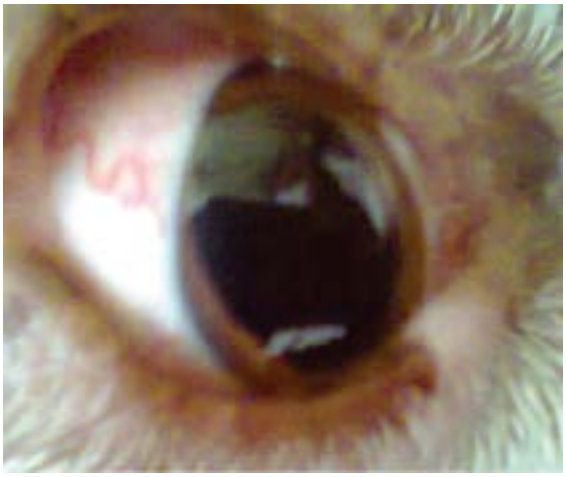

Figure 3a

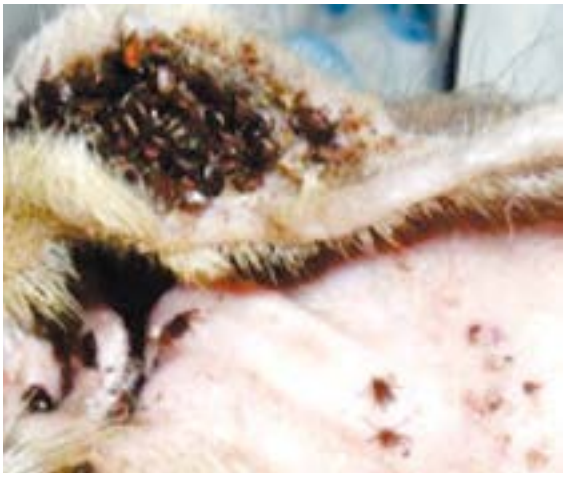

Figure 3b

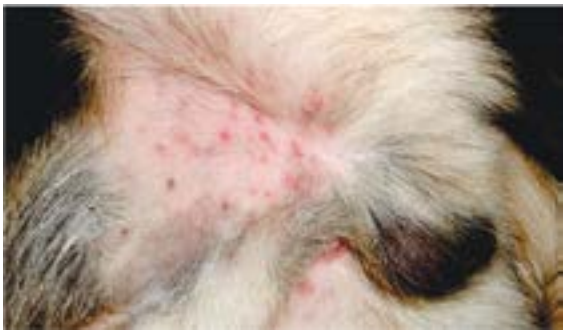

Figure 3c

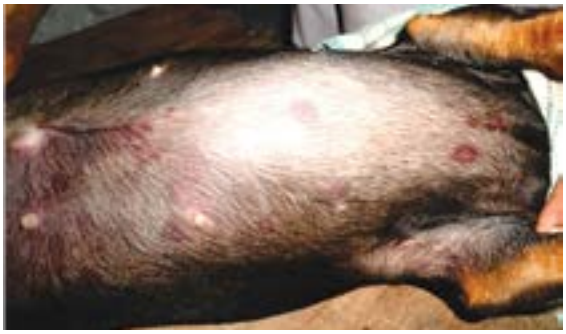

Figure 3d

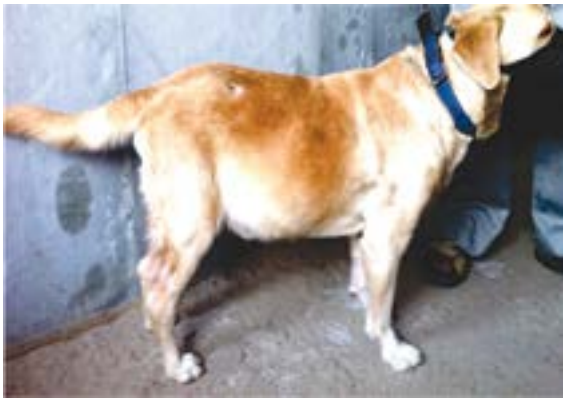

Figure 3e

Figure 3. Clinical findings in Babesia infected dogs:
3a. Ocular bleeding.
3b. Severe tick infestation in pinna
3c. Petechial haemorrhagic spots on inguinal region.
3d. Large circular red spots on ventral aspect of abdomen
3e. Ascites

RESULTS

These 41 clinical cases of canine babesiosis were selected from a total of 964 cases of dogs presented in the clinics, thus presenting an overall prevalence of 4.25 per cent representing 3.84 per cent (37/964) cases of *B. gibsoni* and 0.41 per cent (4/964) of *B. canis*. Of these ailing dogs, 29 were male (70.73%) and 12 were female (29.26%). The affected dogs were of different breeds: Labrador (19), German Shepherd (5), Pug (2), Saint Bernard (2), Rottweiler (2), Dalmatian (2), Pomeranian (2), Daschund (2), Boxer (1), French Mastiff (1), Great Dane (1) and non-descript (2). Diagnosis was based on presence of intra-erythrocytic *Babesia* organism and confirmed by PCR. The most common clinical symptoms observed were anorexia, inappetance, pyrexia, nausea, vomiting and pale mucous membrane. A three-host tick, *Rhipicephalus sanguineus* was the only tick detected in the present study which thrives as the biological vector of canine babesiosis in this part of the world (Figure 3b). Haemoglobinurea was observed only in one *B. gibsoni* positive case. In the present study, rare findings of hind limbs weakness, ascites, jaundice, paraplegia, melena, blindness, ocular bleeding, immune mediated haemolytic anaemia (IMHA) and skin lesions were observed (Figure 3). Ascites was one of the signs in few cases of babesiosis (Figure 3e) and haemoglobinuria was observed only in one infected dog. The findings suggested a large variation in clinical signs in canine babesiosis. Clinical examination revealed elevated rectal temperature (104.37+0.22 ^{o}F), tachycardia (156.59+1.64/min) and polypnoea (60.40+1.97/min).

Significant (p<0.01) decrease in haemoglobin, TEC and packed cell volume (Table 1) indicated severe anaemia. Blood films showed anisocytosis and nucleated erythrocytes indicating regenerative anaemia. Leucocyte count was quite variable. Significant (p<0.01) reduction in the lymphocytes and thrombocytes were observed in *B. gibsoni* infected dogs.

Significant (p<0.05) elevation of bilirubin, ALT, AKP, BUN and creatinine were observed in both *B. gibsoni* and *B. canis* infected dogs. Hypoglycaemia was observed in five dogs infected with *B. gibsoni*.

DISCUSSION

The clinical signs observed in the present study seem to be the result of tissue hypoxia following anaemia and a concomitant systemic inflammatory response syndrome caused by marked cytokine release (Lobetti, 2006). Some rare clinical manifestations as observed in the present study have also been reported previously in clinical cases of babesiosis (Wadhwa *et al.,* 2011). A wide range of inconsistent clinical manifestations might be due to multi systemic effects of disease. Present clinical findings indicated that it is important not to neglect babesiosis in differential diagnosis of ascites. Unlike bovine babesiosis, haemoglobinuria is

Table 1. Haemato-biochemical profile in dogs infected with *B. gibsoni* and *B. canis* (Mean+SE)

Parameters	Control (n=10)	*B. gibsoni* (n=37)			*B. canis* (n=4)		
		Mean ± SE	Min	Max	Mean ± SE	Min	Max
Hb (g/dL)	13.3±0.25	10.59±0.62*	3.1	17.2	7.92±1.19**	4.7	10.4
TLC (10^3/µL)	8.44±0.29	20.46±2.13**	1.78	39.27	10.05±1.76	4.84	12.21
TEC (10^6/µL)	6.5±0.11	5.29±0.32*	1.4	7.9	3.80±0.58**	2.2	4.9
PCV (%)	38.77±1.17	29.40±1.77**	7	49.8	21.12±3.20**	12	27
Platelet (10^5/µL)	3.71±0.28	1.94±0.24**	0.09	7.18	1.14±0.34**	0.52	1.65
Neutrophils (%)	73±0.91	81.13±1.77**	58	97	75±10.66	46	96
Lymphocytes (%)	27±0.91	17.02±1.44**	2	36	23.5±10.53	4	52
Eosinophils (%)	1.5±0.27	1.86±0.68	0	23	2±0.70	0	3
Albumin (g/dL)	3.05±0.07	2.73±0.11	1	4.1	2.57±0.53	1.8	3.3
Globulin (g/dL)	3.92±0.06	3.71±0.15	1.8	5.8	6.37±2.33	2.1	11.8
Albumin: Globulin	0.79±0.05	0.78±0.05	0.41	2.16	0.51±0.18*	0.24	1.06
Total protein (g/dL)	6.32±0.14	6.43±0.20	3	9.1	8.95±2.66	3.1	14.7
Total bilirubin (mg/dL)	0.4±0.05	1.82±0.69*	0.2	19.8	1.12±0.27**	0.5	1.8
ALT (U/L)	27.4±3.40	48.40±9.01*	8	313	58±16.46**	10	83
ALKP (U/L)	81.1±4.99	242.49±38.42*	45	900	165.25±44.53**	60	259
BUN (mg/dL)	15.9±1.81	31.63±7.96*	6	300	46.75±20.06*	13	105
Creatinine (mg/dL)	0.83±0.03	1.70±0.24*	0.1	6.6	2. 35±1.12*	0.6	5.6
Blood glucose (mg/dL)	107.3±2.75	93.10±5.80	20	180	98.25±9.72	83	120

*Significant difference (P<0.05) with control group. **Significant difference (P<0.01) with control group.

rarely seen in canine babesiosis (Gupta *et al.,* 2002; Varshney *et al*., 2008) as was observed only in a single case in the present study. Elevated rectal temperature, tachycardia and polypnoea simulates to the findings of Wadhwa *et al.* (2011). Immunological responses play an important role in the pathogenesis of babesiosis (Singla *et al.,* 2014).

Severe microcytic-hypochromic anemia may have been initiated by antibody mediated cytotoxic destruction of erythrocytes and/or by auto-antibody directed against components of the membranes of infected and uninfected erythrocytes which has also been reported previously in *B. gibsoni* infection (Aysul *et al*., 2013). Further parasitaemia may result in increased osmotic fragility of erythrocytes (Makinde and Bobade, 1994) and serum haemolytic factors (Onishi and Suzuki, 1994) resulting in haemolytic anaemia (Jacoson and Clark, 1994).

Lymphopenia has also been reported earlier in natural cases of canine babesiosis (Shah *et al*., 2011). Significant

(p<0.01) thrombocytopenia could be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation (Boozer and Macintire, 2003).

Significant (p<0.05) elevation of bilirubin, ALT and AKP are indicative of hepatic hypoxia (Aysul *et al*., 2013). Whether the insult is due to inflammatory cytokines, hypoxic damage, or a combination of these is not known. Acute renal failure in canine babesiosis (Schoeman, 2009) might have resulted into significant (p<0.05) increase BUN and creatinine both *B. gibsoni* and *B. canis* infected dogs. Hypoglycaemia could be as a result of sepsis causing anorexia, impaired hepatic function and increase in breakdown in system (Konto *et al*., 2014). According to Amie (2009) increased non-insulin mediated glucose consumption believed to be induced by inflammatory mediators, more especially in macrophage-rich tissues like the spleen, liver and the lungs was the cause of hypoglycaemia in the affected dogs and at the same time regarded it as a poor prognostic indicator.

CONCLUSION

The presumptive diagnosis of canine babesiosis can be made based on a fever, anaemia, anorexia, vomiting and pale mucous membrane. Microscopic examination may not be very revealing in the detection of very low parasitemia, but it can be considered as the most rapid confirmatory method. The main clinico-pathological findings in canine babesiosis indicate that it typically causes haemolytic anemia, neutrophilia, lymphopenia, moderate to severe thrombocytopenia and multiple organ dysfunctions. Elevation of bilirubin, ALT and AKP are indicative of hepatic hypoxia and increase BUN and creatinine are indicative of degenerative changes in kidneys. Results of this study suggest that haemato-biochemical changes could be beneficial in determination of the severity of babesiosis in dogs.

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