Clinical and laboratory findings of *Babesia* infection in dogs

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Abstract Canine babesiosis is a worldwide tick borne disease. Dogs with fever, in appetite and enlarged lymph nodes were screened for the presence of haemoprotozoan parasites during the one year period of study at T.V.C.C., C.V.Sc., Proddatur. Based on the stained peripheral blood smears examination, six dogs were found to be affected with babesiosis. Clinical examination of the dogs revealed ticks over the body, congested conjunctival mucus membranes, dullness, fever, tachycardia, tachypnoea in all the dogs. Some of the dogs had icterus, lymphadenopathy, haemoglobinuria. Laboratory examination of the clinical samples revealed reduction in haemoglobin concentration, erythrocyte count, platelet count, serum total protein, serum albumin and glucose levels. Increased serum SGPT, creatinine, BUN levels were recorded. Dogs were treated with inj. Diminazene aceturate (Berenil RTU) @ 5 mg/kg body weight, single dose along with supportive and symptomatic therapy in individual cases.

Keywords Babesiosis · Clinical signs · Dogs · Laboratory findings

Introduction

Canine babesiosis is a clinically significant and geographically widespread haemoprotozoan disease of domesticated dogs and wild canids (Irwin 2010). The large *Babesia canis* and the small *Babesia gibsoni* are two organisms commonly known to infect the dogs. The life cycle of *Babesia* includes two stages: inside the host RBCs, in which the sporozoites convert into piroplasms, and the other inside the tick vector (Uilenberg 2006). A typical intra erythrocytic piroplasma is pear-shaped and often occurs in pairs (Homer et al. 2000). Clinically, the illness is characterized by fever, pale mucous membrane, anorexia, anemia, icterus, lymphadenopathy and splenomegaly (Boozer and Macintire 2003). Clinical signs are exceedingly variable; the classical presentation is a febrile illness with perceptible anemia. The severity of babesiosis varies from sub clinical infection to extensive organ failure and death. The most common hematologic alterations associated with *B. canis* infections are anemia and thrombocytopenia (Schetters et al. 2009). The parasite has been reported to be transmitted by ixodid tick vectors from different geographical locations. Depending on the species of *Babesia* parasite present in an area, the common tick vectors reported to serve in transmission of canine babesiosis include *Rhipicephalus* species, *Haemaphysalis* species and *Dermacentor* species (Matjila et al. 2004). The parasite also transmitted by blood exchange and trans-placental route can also possible (Jefferies et al. 2007). The objectives of this study were to describe the clinical signs and hemato-biochemical alterations in dogs affected with *Babesia*.

Materials and methods

The present study was performed on dogs presented and referred to the Teaching Veterinary Clinical Complex,

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College of Veterinary Science, Proddatur from November 2012 to November 2013. In this study, a total number of 300 dogs belongs to the different breeds were examined for the presence of *Babesia* in peripheral blood smears. Each suspected dog was subjected to physical and clinical examination. During the clinical examination, the presence of ticks on the coat of dogs were observed and collected for their identification. Peripheral blood was collected from the ear tips of the dogs for Giemsa-stained thin blood smear examination to detect *Babesia* species in RBC. The smears were fixed in methanol for 5 min and stained by 10 % Giemsa. 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 10 % EDTA solution from all the dogs was used for estimation of packed cell volume (PCV), total leucocyte count (TLC), total erythrocyte count (TEC) and haemoglobin (Hb). The peripheral blood smears were stained by Leishman's stain to study the differential count (DLC) by battlement method following which; the absolute counts were also calculated. Thrombocyte count was carried out only in the three seriously affected dogs with babesiosis. Serum was also collected to study the sero biochemical parameters including SGPT, BUN, total protein, serum albumin by using Span diagnostics Ltd. Kits. The procedures followed as per the Sivajothi et al. (2013). Haematological and biochemical findings of the infected dogs were compared with haematobiochemical findings of apparently healthy dogs of the local area (Reddy et al. 2014). Urine samples were collected from the *Babesia* infected dogs for urine analysis by urine dipstick method and microscopic examination of urinary sediment. The data in respect of each parameter was tabulated and statistical analyses were carried out by using Statistical Package for Social Sciences (SPSS) using Student's *t*-test.

Results

Microscopic evaluation of the stained peripheral blood smears demonstrated the presence of typical large pyriform, paired parasites of *Babesia* in the erythrocytes (Fig. 4) of six dogs in this study. Dogs included in this study had 1–8 years age belongs to the both sex. Clinical examination of the six infected dogs revealed presence of ticks over the body (6/6), dullness (6/6), variations in the appetite (6/6), rise in rectal temperature (6/6), tachycardia (6/6), tachypnoea (5/6), dyspnoea (5/6), congested mucus membranes with sunken eye balls (5/6), lymphadenopathy (5/6), pallor (3/6), haemoglobinuria (3/6), tensed abdomen (2/6), yellowish mucus membranes (2/6), yellowish discoloration of abdomen (2/6), vomitions (2/6), diarrhoea

(2/6), edema at the legs (1/6) and constipation (1/6) (Figs. 1, 2, 3). Based on the morphological characteristics, collected ticks were identified as *Rhipicephalus* spp.

Haematological and biochemical findings were incorporated in the Table 1. Significant reduction in RBC, Hb concentration, PCV percentage and platelet count ($p < 0.05$) were recorded among infected dogs when compared to the apparently healthy dogs. Significant decrease in WBC count and neutropenia along with lymphocytosis and monocytosis was noted. Three dogs with *Babesia* infection had lowered thrombocyte count (range:96,000–112,000/cumm, with mean of $105,333 \pm 36,223/\text{cumm}$). Thrombocyte count was compared with standard reference range mentioned by the Rizzi et al. (2010). Serum biochemical parameters revealed reduction in total protein, serum albumin, glucose levels. Increased BUN, creatinine, SGPT levels were noticed. Laboratory urinalysis was done in all the dogs. 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.

Dogs were treated with inj. Diminazene aceturate (Berenil RTU, Intervet) @ 5 mg/kg body single dose IM, inj. Nurobion forte @ 2 ml IM, inj. Meloxicam @ 0.5 mg/kg body weight IM. Symptomatic therapy along with fluids were given based on requirement of the individual case. Supportive therapy was given with four doses of iron dextran (Imferon) @ 2 ml/dog at weekly twice, daily supplementation of oral haematinics (Sharkoferrol, Alem-bic Ltd.). After completion of 2 weeks of therapy, cases (5/6) were responded well and attains its normal activities. But one dog was not responded and died on the 3rd day of therapy.

Fig. 1 Dog showing the congested mucus membranes and sunken eyeballs

Fig. 2 Dog showing the yellowish buccal mucus membrane

Fig. 3 Dog showing the enlarged lymph node

Fig. 4 Giemsa-stained peripheral blood smear of infected dog showing the pear-shaped large *B. canis* inside the RBCs ($\times 1,000$)

Discussion

Babesia normally causes massive injuries to the host depending on the virulence and pathogenicity of the parasite. The extent of the injuries also depends on the age and

the hygiene of the host (Leschnik et al. 2007). Clinical signs observed in the present study includes depression, fever, tachycardia, tachypnoea, in appetite and pallor mucus membranes. It is thought that the clinical signs are the result of tissue hypoxia following the anaemia and a concomitant systemic inflammatory response syndrome caused by marked cytokine release in babesiosis (Lobetti 2006). In the severe form of the disease there is a marked haemolytic anaemia, severe acid–base abnormalities with frequent secondary multiple organ failure and complications such as acute renal failure, hepatopathy with marked icterus, hypoglycaemia (Keller et al. 2004). Schetters, et al. (2009) describes the classical presentation of *Babesia* infection as febrile illness with apparent anemia though the clinical presentation of canine babesiosis can be highly variable. The common laboratory abnormalities in this study was anaemia and thrombocytopenia. *Babesia* initiates a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes and anemia may be more dependent on the host immune response than on the direct destruction of RBC by the piroplasm (Boozer and Macintire 2003). The mechanisms of the thrombocytopenia are not yet fully understood in babesiosis; multiple mechanisms, including platelet sequestration in the spleen, immune-mediated platelet destruction and development of disseminated intravascular coagulation are possible. In spite of thrombocytopenia no single dog in this study was showed any hemorrhages on the surface of the body. In this study, the main biochemical changes were hypoproteinaemia, hypoalbuminemia and hypoglycemia, increased serum levels of SGPT, creatinine, BUN in majority of the infected dogs. This finding concurs with the previous reports (Furlanello et al. 2005). Increased serum activity of transaminases was suggesting that these abnormalities resulted from hepatopathy and renal failure. Centrilobular hepatitis with hypoxic liver damage could be the possible mechanism that resulted in significant changes in hepatic enzymes and decreased albumin levels (Taboada and Lobetti 2006). Renal impairment noticed due to damage of 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 (Zygner and Wedrychowicz 2009). Hypoglycemia in this study was due to in appetite due to sepsis and impaired hepatic function. This finding was in consonant with the report of Amie (2009) who reported that the increased non-insulin mediated glucose consumption is believed to be induced by inflammatory mediators, more especially in macrophage-rich tissues like the spleen, liver and the lungs. Dogs with babesiosis treated with a single intramuscular injection of diminazene aceturate at the dose of 5 mg/kg (Birkenheuer et al. 1999). In dogs affected with babesiosis early diagnosis and treatment, the prognosis is good, but severely affected or untreated animals may die.

Table 1 Haemato-biochemical values of *Babesia* infected dogs (Mean $\pm$ S.E)

Parameters	Normal group\$	<i>Babesia</i> infected dogs ($n = 6$)	Observed range	<i>t</i> -test	<i>p</i>
Hb (g/dl)	13.03 $\pm$ 0.42	9.72 $\pm$ 0.32	10.2–9.2	6.84**	0.000
PCV (%)	39.29 $\pm$ 2.14	30.50 $\pm$ 6.51	33–27	3.52**	0.001
TEC $\times 10^6$ /cumm	6.51 $\pm$ 0.19	4.42 $\pm$ 0.86	4.8–3.8	8.36***	0.000
TLC/cumm	9334.29 $\pm$ 81.34	8786.67 $\pm$ 71.45	9500–8200	2.62*	0.021
Neutrophils/cumm	6698.06 $\pm$ 244.96	3804 $\pm$ 109.26	4200–3216	7.62***	0.000
Lymphocytes/cumm	2417.37 $\pm$ 119.77	4512 $\pm$ 289.41	4960–4100	12.37***	0.000
Eosinophils/cumm	135.57 $\pm$ 22.11	244 $\pm$ 65.74	312–165	1.54	0.153
Monocytes/cumm	68.36 $\pm$ 17.83	220 $\pm$ 88.62	332–140	3.47**	0.005
Total protein (g/dL)	6.81 $\pm$ 0.22	5.48 $\pm$ 0.21	4.8–6.1	4.27*	0.001
Albumin (g/dL)	3.25 $\pm$ 0.04	2.43 $\pm$ 0.09	2.8–2.2	8.29***	0.000
Globulin (g/dL)	3.56 $\pm$ 0.20	3.05 $\pm$ 0.49	3.9–2.4	1.76 ^{NS}	0.104
A:G ratio	0.85 $\pm$ 0.11	0.79 $\pm$ 0.10	1.16–0.56	1.38 ^{NS}	0.196
Glucose (mg/dL)	120.80 $\pm$ 6.22	88.83 $\pm$ 5.70	72–110	3.73**	0.003
SGPT (ALT) (U/L)	18.71 $\pm$ 1.56	53.00 $\pm$ 8.2	92–38	3.20**	0.008
BUN (mg/dL)	16.56 $\pm$ 0.95	25.67 $\pm$ 2.55	32–24	3.77**	0.003
Creatinine (mg/dL)	0.91 $\pm$ 0.07	1.37 $\pm$ 0.14	1.8–0.8	2.87*	0.015

\$ (Reddy et al. 2014)

NS non significant ($p > 0.05$)* Significant ($p < 0.05$)** Highly significant ($p < 0.01$)

Based on the present study, dogs with fever, lymphadenopathy, inappetence, pale mucous membrane associated with anemia and thrombocytopenia can be suspected for *Babesia* infection. All the suspected dogs in this study were diagnosed only based on the microscopic examination of the stained peripheral blood smears. Microscopic examination may not detect low parasitemia though; it remains the most rapid confirmatory method which was carried out in the present study.

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