HAEMATO-BIOCHEMICAL CHANGES IN DOGS INFECTED WITH *BABESIA* SP.

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The aim of the presented study was to statistically analyze haemato-biochemical abnormalities in *Babesia* sp. infected dogs. Sixty-eight dogs presented to Small Animal Medicine Unit of Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Institution and Research with clinical signs suggestive of *Babesia* sp. were subjected for blood smear examination, from which nine dogs were positive for babesiosis. Dogs with babesiosis were subjected for various haemato-biochemical studies and the data obtained was analyzed statistically. Even though drastic changes were noticed in individual dogs, the most significant abnormality was found to be thrombocytopenia.

Key words: Babesiosis, Blood analysis, Dogs, Thrombocytopenia

Canine babesiosis is caused by haemoprotozoa of the genus *Babesia* (Ayoob *et al.*, 2010) which are apicomplexa protozoa that infect a variety of domestic and wild animals with worldwide distribution and global significance (Laia and Beneth, 2011; Beugnet and Moreau, 2015). Multiple species of *Babesia* have been documented to infect dogs with babesio-

sis. However, *Babesia* sp. are highly prevalent species found in India. Dogs infected with *Babesia* sp. were generally presented with acute regenerative haemolytic anemia and thrombocytopenia (Laia and Baneth, 2011). *Babesia* sp. causes acute regenerative haemolytic anemia (Vial and Gorenflot, 2006), which is usually normochromic to hypochromic in dogs (Taboada,

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1998), immune mediated and thrombocytopenia along with leukocytic changes, influenced by stress, systemic inflammation and bone marrow stimulation (Birkenheuer *et al.*, 1999; Yamane *et al.*, 1993; Laia and Baneth, 2011). Besides severe anemia, lack of severe bilirubinemia is considered a typical sign of *Babesia* sp. infection in dogs. Tissue hypoxia, hypotensive shock and cardiac dysfunction were also observed (Ayoob *et al.*, 2010). There are no studies from Puducherry concerning haemato-biochemical abnormalities in *Babesia* sp. infected dogs. The present study was conducted to perform thorough haematological and biochemical evaluation in *Babesia* infection, analyze and interpret the data statistically.

MATERIALS AND METHODS

The dogs presented to Small Animal Medicine Unit of Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry with clinical signs suggestive of *B. gibsoni* infection were selected for the present study. The dogs which were less than 3 years of age were subjected for blood smear examination and positive cases were subjected for detailed blood analysis. Six milliliters of blood from the tested positive dogs were collected from the saphenous / cephalic vein and 3 mL was dispensed into ethylenediaminetetraacetic acid (EDTA) vacutainer and the remaining was kept for serum separation and were subjected for

various haematological parameters and serum biochemistry respectively. Six apparently healthy dogs brought for vaccination / deworming / routine clinical checkup were selected to form control group and haemato-biochemical values obtained from control group were compared to the diseased group and analyzed.

In the present study, haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC) counts, white blood cell (WBC) counts, platelet count and absolute differential leucocyte counts (DLC) the neutrophil, eosinophil, lymphocyte, monocyte and basophil values using the standard methods (Jain, 1986) were analysed. The erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were calculated using standard formulae (Schalm *et al.*, 1975). From the serum samples, Glucose, BUN, creatinine, ALT, AST, bilirubin, total proteins, albumin, globulin and creatine kinase were analyzed both in control and disease group as well and were interpreted.

For statistical analysis, GraphPad Prism – 5 software was employed to compare the mean values between control and diseased groups with unpaired t-test as described by Snedecor and Cochran (1994) and discussed.

Table 1. Serum biochemistry of apparently healthy dogs and dogs with babesiosis

Parameter	Apparently healthy dogs (n=6)	Dogs with babesiosis (n=9)	Min.	Max.	P value
Hb (g/dL)	13.12 ± 0.47	10.16 ± 1.31	3.5	14.9	0.099 ^{NS}
PCV (%)	39.52 ± 1.24	29.80 ± 3.825	10.8	40.6	0.0665 ^{NS}
RBC (× 10 ⁶ /mm ³)	5.9 ± 0.2	4.59 ± 0.34	3.06	5.97	0.013*
MCV (fl)	67.17 ± 2.3	64.71 ± 6.58	26.43	84.49	0.711 ^{NS}
MCH (pg)	22.32 ± 0.96	21.79 ± 2.2	10.57	29.08	0.85 ^{NS}
MCHC (%)	33.20 ± 0.61	34.35 ± 1.017	29.68	40	0.4098 ^{NS}
WBC (10 ³ /mm ³)	8.46 ± 0.74	12.89 ± 4.809	3.6	50.5	0.47 ^{NS}
Neutrophils (10 ³ /mm ³)	5.754 ± 0.588	8.61 ± 2.26	2.08	25.27	0.33 ^{NS}
Lymphocytes (10 ³ /mm ³)	2.517 ± 0.340	3.09 ± 1.70	0.59	16.68	0.78 ^{NS}
Monocytes (10 ³ /mm ³)	0.095 ± 0.035	0.32 ± 0.15	0.048	1.51	0.26 ^{NS}
Eosinophils (10 ³ /mm ³)	0.08 ± 0.04	0.16 ± 0.9	0	0.67	0.4648 ^{NS}
Platelet (10 ⁵ /mm ³)	3.382 ± 0.35	1.523 ± 0.34	0.25	3.7	0.0032**
Glucose (mg/dL)	90.5 ± 3.5	75.67 ± 4.4	48	98	0.032*
BUN (mg/dL)	22.50 ± 1.945	22.95 ± 2.36	10	35	0.894 ^{NS}
Creatinine (mg/dL)	0.81 ± 0.15	0.84 ± 0.08	0.5	1.2	0.9 ^{NS}
ALT (IU/L)	35.17 ± 2.25	53.78 ± 5.82	24	85	0.026*
AST (IU/L)	46.83 ± 5.73	57.56 ± 7.21	29	110	0.305 ^{NS}
Bilirubin (total) (mg%)	0.5 ± 0.02	0.66 ± 0.17	0.4	2	0.473 ^{NS}
Direct bilirubin (mg%)	0.11 ± 0.01	0.21 ± 0.09	0.1	1	0.459 ^{NS}
Indirect bilirubin (mg%)	0.38 ± 0.01	0.44 ± 0.07	0.3	1	0.5043 ^{NS}
Total protein (g/dL)	6.23 ± 0.125	5.6 ± 0.243	4.4	6.5	0.081 ^{NS}
Albumin (g/dL)	2.41 ± 0.17	2.21 ± 0.13	1.6	2.7	0.362 ^{NS}
Globulin (g/dL)	3.81 ± 0.10	3.42 ± 0.17	2.6	4.19	0.1190 ^{NS}
Albumin : Globulin	0.63 ± 0.05	0.65 ± 0.04	0.45	0.86	0.8190 ^{NS}
Creatine kinase (U/L)	172.3 ± 33.81	231.4 ± 43.99	95	462	0.3489 ^{NS}

**: Significant (P ≤ 0.01); *: Significant (P ≤ 0.05); NS: Not Significant (P > 0.05)

RESULTS

In the present study, sixty-eight dogs were suspected for *Babesia* sp. infection and out of which nine were found to be tested positive. Whole blood and serum samples collected were subjected for various hematological and biochemical analysis (Table 1) and the mean values were compared with the control group. Haematology of the infected group showed significant difference in mean RBC and platelet counts when compared to the control group. RBC and platelet counts were significantly lower with $P < 0.05$. In serum biochemistry, significant reduction in mean value of glucose (75.67 ± 4.4 mg/dL) and increase in mean value of ALT (53.78 ± 5.82 IU/L) were observed in the dogs with babesiosis when compared to the control group.

DISCUSSION

Anemia and thrombocytopenia were the only significant findings noticed in the present study and the findings correlate with the reports of Birkenheuer *et al.* (1999) and Ayoob *et al.* (2010), who reported that acute hemolytic anemia with concurrent thrombocytopenia were present in most of the cases affected with babesiosis. Thrombocytopenia was the consistent finding in all the cases affected with babesiosis (Fig. 1). Even though there was no significant difference noticed in haemoglobin and PCV values in the diseased

group, three out of nine dogs were affected with severe extravascular hemolysis with haemoglobin levels less than 8.0 mg/dL, the lowest value being 3.5 mg/dL. Anisocytosis was noticed in few dogs (Fig. 2) which is a common finding in canine babesiosis besides anemia (Zygner *et al.*, 2007; Ombowale *et al.*, 2017)

In the present study elevation in mean alanine aminotransferase (ALT) was noticed, which is incongruence with Aysul *et al.* (2013) and there is decrease in mean glucose levels and the result is in acceptance with Konto *et al.* (2014). Hypoglycemia in canine babesiosis was reported by various authors (Amie, 2009; Ayoob *et al.*, 2010; Konto *et al.*, 2014). Aysul *et al.* (2013) reported that in dogs suffering from babesiosis, significant elevation of bilirubin, ALT and alkaline phosphatase levels could be seen with hepatic hypoxia. Reasons behind hypoglycemia in canine babesiosis were postulated as increased glucose consumption secondary to anaerobic glycolysis, hyper metabolism and enhanced cellular uptake of glucose, depletion of hepatic glycogen stores and hepatic dysfunction with impaired gluconeogenesis (Ayoob *et al.*, 2010).

Even though there was a significant reduction in blood glucose levels and increase in ALT levels, the values were still within the normal range.

Anemia and thrombocytopenia were the only consistent haematological

Haemato-biochemical changes in dogs infected with *Babesia* sp.

Fig. 1. Box plot showing variation in platelet counts between diseased and control groups

Fig. 2. Blood smear showing anisocytosis

abnormalities found in *Babesia gibsoni* infected dogs. Even though anemia was significant in the present study, only three out of nine dogs suffered from anemia which shows varied presentation of the blood picture in infected dogs. Serum biochemical analysis showed very few and insignificant changes.

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