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Haemato-biochemical profile of three dogs naturally infected with *Hepatozoon canis*

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Abstract
To evaluate clinical and hematological aspects of dogs naturally infected with Hepatozoon canis (H. canis) presented at the TVCC, Department of Veterinary Medicine, LUVAS, the present study was performed. Three dogs were found positive on microscopic blood smear examination and the clinical symptoms were mild to moderate fever, pale mucosae and lethargy and one dog was showing vomiting symptoms. Haematological alterations showed increased MCV and monocyte count. Serum biochemical values showed increase in AST, GGT and ALP.

Keywords: *Hepatozoon canis*, dogs, hematobiochemical changes

Introduction
Hepatozoonosis, an enzootic haemoprotozoan disease with a variable prevalence, is caused by several species of Hepatozoon, a genus in the phylum Apicomplexa, suborder and family Hemogregarinidae (Hepatozoidae). Hepatozoon species infect a wide variety of domestic and wild animals. *Hepatozoon canis* (*H. canis*) infection among dogs is widespread in Africa, South Europe, South America and Asia. H. canis, firstly reported in India (James 1905), is the cause of old world canine hepatozoonosis, which generally leads to a mild disease that affects the spleen, lymph nodes and bone marrow, resulting in anaemia and lethargy. The disease is transmitted by the ingestion of definitive host of *H. canis*, the brown dog tick, *Rhipicephalus sanguineus* (Beneth *et al.*, 2003). The present study was conducted in LUVAS, Department of Veterinary Medicine to describe the clinical and haematological findings of dogs founded infected by *Hepatozoon canis*, in view of the lack of such information about this tropical disease in Haryana state.

Materials and Methods
A complete clinical history of suspected dogs including breed, age, sex, body weight, type of diet given, nature of illness, clinical signs of haemoparasitic infection, any previous treatment given etc. were collected from the pet owner and were recorded. After taking the clinical history, the detailed clinical examination of dogs in terms of measurement of vital parameters and complete haemato-biochemical analysis, was done and the observations were recorded. The clinical vital parameters recorded were rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute). About 5 ml blood was collected from cephalic/saphanous vein by using 22G S/V set and 5 ml disposable syringe aseptically. One ml of blood was poured into two tubes coated with K_3 ethylenediamine-tetraacetic acid (K_3EDTA) separately for hematological examination. Four ml blood was poured in plain tube (without anticoagulant) with clot activator for harvesting serum. The blood samples collected in plain tube were kept undisturbed for 2 hours and then, centrifuged at 3000 rpm for 5 minutes and the serum separated were decanted in 2 ml Eppendorf tubes. The blood samples collected in tubes coated with K_3EDTA were immediately analyzed for complete hematological examination using fully automated Heamatology cell counter (MS4s, Melet Schlosing Lab.). The erythrocytic indices measured were haemoglobin (Hb) in g/dl, total erythrocyte count (TEC) in M/mm^3,hematocrit (Hct) in %, mean corpuscular volume (MCV) in fl, mean corpuscular hemoglobin (MCH) in pg and mean corpuscular hemoglobin concentration (MCHC) in g/dl. The leucocytic indices measured were total leucocyte count (TLC) in m/mm^3, lymphocytes (L) in %, monocytes (M) in %, neutrophils (N) in %, eosinophils (E) in % and basophils (B) in %. The thrombocytic indices measured were thrombocyte count (THR) in m/mm^3, mean platelet volume (MPV) in fl and plateletcrit (Pct) in %.

Thin blood smears from the micro-capillary circulation (ear tip) in duplicate from each suspected cases were prepared on clean, grease free microslides, air dried and fixed using methanol. The fixed blood smears were stained by Giemsa stain using 1:10 dilution for 30 – 40 minutes (Coles, 1986). The slides were washed under running tap water, air dried and examined microscopically at 1000 times magnification under oil immersion for *Hepatozoon canis*. The serum samples were analyzed for estimation of biochemical profile using fully automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). The serum biochemical parameters of liver function measured were alanine aminotransferase (ALT) in U/L, aspartate aminotransferase (AST) in U/L, gamma glutamyl transferase (GGT) in U/L, bilirubin (total) in mg/dl, bilirubin (direct) in mg/dl, bilirubin (indirect) in mg/dl and alkaline phosphatase in U/L. The protein profile included the estimation of total protein in g/dl, albumin in g/dl, globulin in g/dl and albumin to globulin ratio (A/G). The serum biochemical parameters of kidney function measured were urea in mg/dl and creatinine in mg/dl. The lipid metabolites measured were triglycerides in mg/dl and total cholesterol in mg/dl.

Results and Discussion

Three dogs were diagnosed for hepatozoonosis based upon characteristic ellipsoidal shaped gamonts of *Hepatozoon canis* inside neutrophils in stained blood smear examination (Fig.1). Breeds affected were Labrador Retreiver, German Shepherd and Spitz. Predominance of male dogs was found in *Hepatozoon canis* infection.With regard to age, it was found that the examined dogs were more than one year age. Anorexia, mild to moderate fever, weight loss and vomiting were common clinical signs observed in affected dogs. The examination of vital parameters revealed significant (P<0.05) increase in temperature in affected dogs as compared to healthy control. Pulse rate and respiration rate altered but not significantly as depicted in table 1.

Haematological examination revealed significant (P<0.05) increase MCV and monocytes count in dogs affected with *Hepatozoon canis* as compared to healthy control. No significant difference was observed in mean values of other parameters in affected dogs in comparison to healthy control as depicted in Table 2. Serum biochemical profile revealed significant rise in mean values of AST, GGT and alkaline phosphatase and significasnt increase in total bilirubin levels with rest of the parameters non-significantly altered in affected dogs than those of healthy control as depicted in table 3.

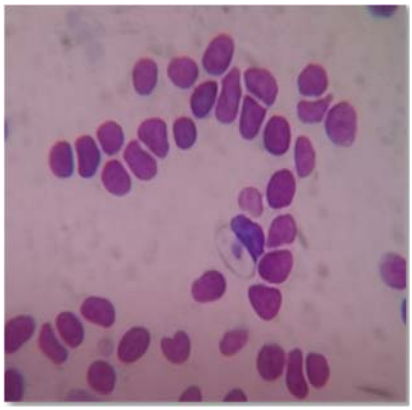

Fig 1: Gamont of *Hepatozoon canis* in Neutrophil in blood smear examination

Table 1: Vital parameters of dogs affected with *Hepatozoon canis* (Mean ±S.E.)

Parameters	Healthy Control (n=6)	Affected animals(n=3)
Temperature (°F)	102.12±0.24	103.49±0.42*
Pulse rate (per minute)	87.00±4.46	92.00±1.32
Respiration rate (per minute)	36.44 ±1.89	41.20±1.24

Value with superscript * differ significantly (P<0.05) in a row

Table 2: Hematological profile of dogs diagnosed with *Hepatozoon canis* (Mean ±S.E.)

Parameters	Healthy Control (n=6)	Affected animals (n=3)
Hb (g/dl)	10.83±0.73	9.73±0.74
TEC (M/mm^3)	5.58 ±0.40	4.62±0.83
Hct (%)	32.56±1.98	30.33±0.55
MCV (fl)	58.61±1.09	68.99±2.78*
MCHC (g/dl)	33.19±0.37	26.36±1.40
MCH (pg)	19.43±0.17	16.36±0.90
TLC (m/mm^3)	10.62±1.41	7.17±1.70
L (%)	25.43±1.29	34.56±2.63
N (%)	71.51±1.58	58.80±2.88
M (%)	2.23±1.02	6.00±0.86*
E (%)	0.60±0.38	0.30±0.02
B (%)	0.21±0.14	0.43±0.14
Thrombocytes (m/mm^3)	411.17±19.50	405.00±12.58
MPV (fl)	7.33±0.17	7.23±0.13
Pct (%)	0.26±0.04	0.21±0.01

Value with superscript * differ significantly (P<0.05) in a row

Table 3: Serum biochemical profile of dogs diagnosed with *Hepatozoon canis* (Mean ±S.E.)

Serum biochemical parameter	Healthy Control (n=6)	Affected animals (n=3)
ALT (U/L)	28.13±2.03	20.46±2.59
AST (U/L)	18.68±2.61	32.56±11.55 *
GGT (U/L)	12.27±2.09	15.56±4.42*
Bilirubin total (mg/dl)	0.27 ±0.02	0.42±0.05 *
Bilirubin direct (mg/dl)	0.16±0.01	0.29±0.01
Bilirubin indirect (mg/dl)	0.11±0.01	0.13±0.01
Alkaline phosphatase (U/L)	142.33±37.55	150.33±12.44*
Total Protein (g/dl)	6.78±0.37	3.86±0.07
Albumin (g/dl)	3.18±0.20	1.99±0.22
Globulin (g/dl)	3.59±0.24	3.91±0.29
A/G ratio	0.89±0.07	0.60±0.27 *
Triglycerides (mg/dl)	105.67±13.80	90.30±15.28
Total cholesterol (mg/dl)	219.61±23.35	158.33±5.92
Urea (mg/dl)	28.93±4.52	48.66±2.53*
Creatinine (mg/dl)	1.81±1.16	1.25±0.32

Value with superscript * differ significantly (P<0.05) in a row

Anorexia, mild to moderate fever, weight loss and vomiting were common clinical signs observed in affected dogs. The examination of vital parameters revealed increase in temperature in affected dogs as compared to healthy control. In *Hepatozoon canis* infection, males preponderated in the study group. These were in agreement with Chabra *et al*. (2013). Haematological examination revealed increase MCV and monocytes count in dogs affected with *Hepatozoon canis* as compared to healthy control. Serum biochemical profile revealed rise in mean values of AST, GGT and alkaline phosphatase and increase in total bilirubin levels with rest of the parameters non-significantly altered in affected dogs than those of healthy control. These findings were in agreement of the workers (Sarma *et al*., 2012) [4]. According to Kerr (2002), increased activities of serum AST, ALT and ALP may be due to liver damage, while increased AST and CK concentrations

were thought to be linked to muscle tissue damage. Gavazza *et al*. (2003) [6] and Sarma *et al*. (2012) [4] observed elevation of ALP in H. canis infection. Elevation of ALP seen in this report might be due to progression of schizogony within bone-morrow and hepatocytes, in addition to the spleen. In this report, the complete blood count revealed normocytic and hypochromic anaemia with neutrophillia as observed by Paramjit *et al*. (2012) [3] in a case of hepatozoonosis in a mongrel dog. Ruiz *et al*. (2013) [2]. These alterations may also be due to polyclonal gamopathy caused by *Hepatozoon canis* (Baneth and Weigler, 1997) [1].

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