



CLINICO-HEMATOLOGICAL CHANGES IN DOGS WITH HEPATOZOONOSIS OF PUDUCHERRY REGION

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ABSTRACT

The aim of this study was to evaluate the clinical and hematological changes in canine hepatozoonosis. Dogs exhibiting clinical manifestations of fever, inappetance, pale to congested mucous membrane and enlarged lymph node along with tick infestation were screened for hepatozoonosis, and the blood smear positive for *Hepatozoon canis* was included in the study group and was compared with the control group. The hematological parameters showed significant decrease ($p < 0.05$) in mean Hemoglobin, Packed cell volume and Red Blood Cell count. The Mean Corpuscular Volume and Total Leukocyte Count, were significantly increased ($p < 0.05$) in *Hepatozoon* affected dogs when compared with the control mean. The mean absolute eosinophil count and the mean absolute neutrophil count showed significant increase compared to control group ($P < 0.01$). Ticks were collected from the *Hepatozoon* infected animals and examined for species identification.

KEYWORDS: *Hepatozoon canis*, hemogram, dogs, ticks.

INTRODUCTION

Hepatozoonosis is a tick-borne disease caused by hemogregarian apicomplexan protozoa in dogs and wild carnivores. The two species are *Hepatozoon canis* and *Hepatozoon americanum*. *H. canis* is transmitted by the brown dog tick, *Rhipicephalus sanguineus* whereas *H. americanum* is transmitted by *Amblyoma maculatum*.^[1,2] Unlike many vector-borne protozoan and bacterial pathogens that are transmitted by the tick bite, *Hepatozoon* infection in dogs is transmitted by ingestion of the infected ticks by the dogs. Hepatozoonosis varies from being asymptomatic in apparently healthy dogs to a severe, life-threatening disease in animals with extreme lethargy, cachexia and anemia.^[3] *Hepatozoon canis* affects blood, vascular and lymphatic tissues as well as hemopoietic organs.^[4] The clinico-pathological changes of canine hepatozoonosis include elevated leukocyte count, mild normocytic, normochromic regenerative anaemia, thrombocytosis, or thrombocytopenia.^[5,6] Diagnosis is based on history of tick infestation, clinical findings, laboratory investigations and serological test. Diagnosis is usually made on the identification of gamonts in the cytoplasm of neutrophils (more rarely monocytes) in blood smear or buffy coat smear.^[7] Due to the lack of relevant information available on clinico-hematological parameters in hepatozoonosis in Puducherry region the current study has been undertaken.

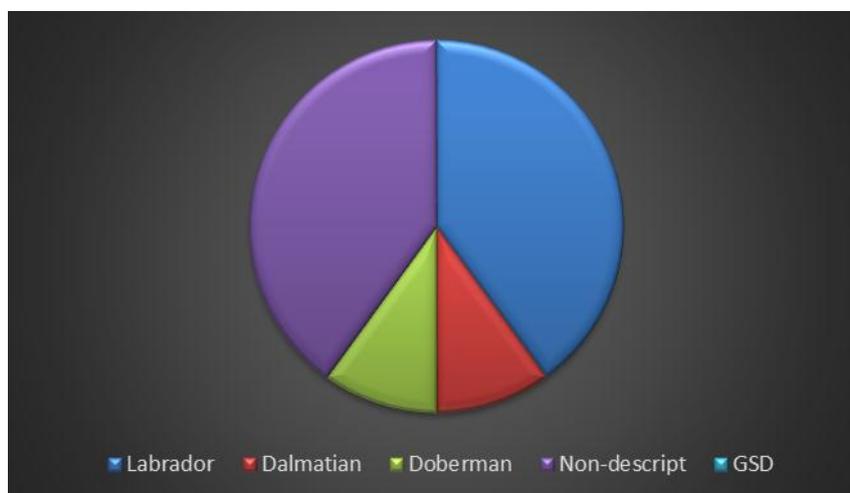
MATERIALS AND METHODS

The present study was taken up at Small Animal Medicine Unit, Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry. Twelve dogs that were apparently healthy and free from blood parasites were taken as control group. The dogs presented with clinical signs such as fever, inappetance, pale mucous membrane and enlarged lymph node along with the history of tick infestation were screened for the blood parasites. Peripheral blood smear was taken from the ear pinna and stained using Leishman's stain and examined under oil immersion objective of the microscope. Dogs turned positive for *H. canis* was included in the study group. Whole blood samples were collected from cephalic/saphenous vein in EDTA vacutainers and evaluated for hematological parameters such as Packed Cell Volume (PCV), Hemoglobin concentration (Hb), red blood cell (RBC) count, total leukocyte count (TLC) and differential leukocyte count using the standard method.^[8] The erythrocytic indices such as Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) values were calculated using standard formulae.^[9] The ticks collected from dogs were identified as per the morphology described by Sen and Fletcher (1962). The data of the control group and the study group were subjected to statistical analysis such as mean, standard deviation and paired 't' test to study significance.

RESULT**Age and Breed**

In the present study, the dogs were categorized into less than a year, 1-5 years age and more 5-year age group. Out of twelve dogs, 4 dogs (33.33%) were less than 1 year age group, 5 dogs (41.67%) were between 1–5-year age group, 3 dogs were above 5-year age group.

Various breeds of dogs like Labrador (4), Dalmatian (1), Doberman Pinscher (1), German shepherd (1), Spitz (1) and non-descript (4) were affected (Fig 1). Of these twelve dogs, seven were male and eight were female.



“Fig.1” Pie-diagram depicting the breed distribution.

Clinico-hematological evaluation**Clinical signs**

The various clinical manifestations in dogs with hepatozoonosis were illustrated in Table 2. The predominant clinical signs were tick infestation (100%),

inappetance (100%), fever (100%), pale mucous membrane (58.3%), congested mucous membrane (41.67%), swollen lymph node (75%) and loss of body weight (66.67%).

Table 2: Clinical signs of dogs with Hepatozoonosis.

S.NO.	CLINICAL SIGNS	NO. OF ANIMALS	PERCENTAGE (%)
1	Inappetance	12	100
2	Fever	12	100
3	Pale mucous membrane	7	58.33
4	Congested mucous membrane	5	41.67
5	Swollen lymph node	9	75
6	Loss in body weight	8	66.67

Hemogram

Erythron and leukogram of apparently healthy dogs and dogs infected with *Hepatozoon canis* were given in Table 3. Erythron of apparently healthy dog in the present study showed a mean Hemoglobin (Hb) of 13.66±0.97 g/dl, mean PCV of 40.92±3.66%, mean RBC count of 6.81±0.65×10⁶ cells/μL, mean MCV of 60.24±2.84 fl, mean MCH of 20.17±1.89 pg/ml and mean MCHC of 33.53±2.54 g/dl. The leukogram revealed the mean leukocyte count of 12.5±4.04×10³ cells/μL.

significantly higher than the control group ($P < 0.05$). The mean absolute eosinophil ($1.01 \pm 1.02 \times 10^3$ cells/μL) count and the mean absolute neutrophil count ($11.46 \pm 3.96 \times 10^3$ cells/μL) showed significant increase compared to control group ($P < 0.01$). The animals were showing macrocytic normochromic anemia, leukocytosis, eosinophilia and neutrophilia.

The mean PCV (29.12±9.97%), Hemoglobin (9.76±3.28 g/dl) and RBC ($4.46 \pm 1.30 \times 10^6$ cells/mm³) of dog with hepatozoonosis were significantly less ($P < 0.05$) when compared with their respective control groups. The mean MCV (64.68±6.98 fl) and TLC ($16.08 \pm 5.58 \times 10^3$ cells/μL) of dog with hepatozoonosis was

Table 3: Hemogram of apparently healthy dogs and dogs with hepatozoonosis.

Parameter	Apparently healthy dogs (n=12)	Dogs with Hepatozoonosis (n=12)	t-test
Hemoglobin (g/dl)	13.66±0.97	9.76±3.28	0.00**
PCV (%)	40.92±3.66	29.12±9.97	0.00**
RBC count ($\times 10^6$ cells/ μ L)	6.81±0.65	4.46±1.30	0.00**
MCV (fl)	60.24±2.84	64.68±6.98	0.03**
MCH (pg/ml)	20.17±1.89	22.27±5.46	0.11 ^{NS}
MCHC (g/dl)	33.53±2.54	34.89±9.79	0.32 ^{NS}
TLC ($\times 10^3$ cells/ μ L)	12.5±4.04	16.08±5.58	0.04**
Absolute Neutrophil Count ($\times 10^3$ cells/ μ L)	7.63±3.17	11.46±3.96	0.008**
Absolute Eosinophil Count ($\times 10^3$ cells/ μ L)	0.052±0.078	1.01±1.02	0.001**
Absolute Lymphocyte Count ($\times 10^3$ cells/ μ L)	4.74±2.5	3.52±2.94	0.14 ^{NS}
Absolute Monocyte Count ($\times 10^3$ cells/ μ L)	0.062±0.092	0.082±0.173	0.36 ^{NS}

Note: ** Significant ($P \leq 0.01$); * Significant ($P \leq 0.05$); NS- Not Significant ($P \geq 0.05$)

Tick identification

The ticks collected from the animals were classified based on morphological features and confirmed as *Rhipicephalus sanguineus*.

Diagnosis

Blood smear examination

Peripheral blood smear from the dog was subjected to Leishman staining. Intracellular ellipsoidal gamonts were noticed in the neutrophils (Fig 1)



“Fig.1”: Blood smear showing Ellipsoid Gamont of *H. canis* in neutrophil.

DISCUSSION

Canine hepatozoonosis is most prevalent in various regions of India^[13] caused due to *H.canis*.^[10-12] In the present study, highest incidence of hepatozoonosis was observed in labrador and Non-descript (33.33%) followed by Doberman (8.33%), Dalmatian (8.33%), Spitz (8.33%) and German shepherd (8.33%). In the present study, female dogs outnumbered males with a ratio of 1.14:1 with a mean age of 3.9 years and is in dissonance with Greene and Jittapalapong *et al.*, who stated that there is no gender or breed predilection for *H.canis*. dogs between 1-5 year age group had the highest incidence. The animal affected with the least age was 45 days and peak age was 11 years which indicates that hepatozoonosis can occur in all age groups and the young ones being affected can be attributed due to low immunity.^[15]

In *H. canis* infection, most of the time the animal may not show any specific clinical signs rather they may show some non-specific clinical signs such as weakness, myalgia, fever and generalized wasting or weight loss.^[3,6,16] whereas in the present study predominant clinical signs noticed were inappetance, fever, pale to congested mucous membrane, swollen palpebral lymph node and loss of body condition.

On hematological analysis, macrocytic normochromic anemia, leukocytosis, neutrophilia and eosinophilia were noticed in dogs with hepatozoonosis compared to apparently healthy dogs. The low hemoglobin can be insinuated towards either deficiency of iron or reduced synthesis of proteins due to progressive damage of hepatocytes following *H. canis* infection. The parasite *H. canis* is believed to affect the liver and spleen.^[17,18] Moreover, the animals were showing macrocytic normochromic anaemia which is in contrary to the authors who stated that normocytic normochromic anaemia was the consistent finding.^[5,6] Neutropenia was also noticed in some rare cases in accordance with Shimoka *et al.*,^[19]

CONFLICT OF INTEREST STATEMENT:

We declare that we have no conflict of interest.

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