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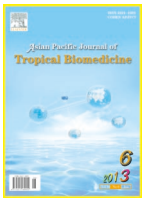

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Retrospective study of clinical and hematological aspects associated with dogs naturally infected by *Hepatozoon canis* in Ludhiana, Punjab, India

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PEER REVIEW

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Comments

This report is a good retrospective study in a large number of cases describing clinical and hematological findings, which is of use for clinicians in diagnosis of the condition. The data are interesting and enlightening other researchers focus on further investigations.
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ABSTRACT

Objective: To evaluate clinical and hematological aspects of dogs naturally infected with *Hepatozoon canis* (*H. canis*) presented at the Small Animal Clinics of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. **Methods:** Blood films of 34 naturally infected dogs were examined for haematological alterations and parasitaemia. Signalment and clinical signs were recorded from the animals. Clinical histories were filled out during the consultation. **Results:** Of the 34 positive dogs by Giemsa stained peripheral blood films, 88.23% presented parasitaemia by *H. canis* only, while 11.77% had the combination of *H. canis*, *Babesia* sp. and *Ehrlichia* sp. Young male dogs less than one-year-old, of non-descript breed, were the most commonly affected. And 26.47% were presented with anorexia/inappetence as the only clinical symptom. Other clinical symptoms were mild to moderate fever, pale mucosae and lethargy; a few were also showing the signs of vomiting and diarrhoea. Haematological alterations showed mainly normochromic-normocytic anaemia, leukocytosis and neutrophilia. **Conclusions:** The findings of this study substantiate that *H. canis* caused clinical and haematological alterations of the varied intensity in dogs, even with low parasitaemia, should be taken into consideration.

KEYWORDS

Clinical signs, Dogs, Haematology, *Hepatozoon canis*

1. Introduction

Hepatozoonosis, an enzootic haemoprotozoan disease with a variable prevalence, is caused by several species of *Hepatozoon*, a genus in the phylum Apicomplexa, suborder Adeleorina 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[1], 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*, and animals from neonatal to adult age which are

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infected[2]. The host range of *H. canis* in carnivores is not yet elucidated, but the presence of *Helicobacter felis* and *H. canis* in both domestic and wild animals suggest that they are not host specific and the same parasite causes infection in domestic and wild felids and canids in different parts of the world[3].

The species found in India is *H. canis*, which is prevalent in several regions of the country[4-6]. Infection of *H. canis* varies from asymptomatic cases in apparently healthy dogs to severe and potentially fatal cases of the disease with variable signs including fever, paralysis, anorexia, emaciation, anaemia, ocular discharge and hind leg weakness[7]. In hepatozoonosis caused by *H. canis*, clinical and laboratory findings are often masked by the presence of concomitant infections, and such findings should not be attributed exclusively to *H. canis*. The purpose of this study is to retrospectively describe the clinical and haematological findings of dogs naturally infected only by *H. canis* and associated concurrently with other agents, in view of the paucity of such information about this tropical disease in dogs in Punjab state.

2. Materials and methods

This study involved 34 dogs of both sexes, of different breeds and ages. The dogs, which displayed varied clinical symptoms, were presented at the Small Animal Clinics of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana from 2009 to 2011. The dogs were diagnosed as naturally infected by *H. canis*, based on the gametocytes found inside the polymorpholeukocytes in the Giemsa stained blood films prepared with blood taken from marginal ear veins.

For the haemogram, about 2 mL of blood was collected from each animal by cephalic vein puncture with disposable needles and syringes. The blood samples were stored in refrigerator in sterile tubes containing K3 EDTA solution. Complete blood count was carried out on fully automated analyzed ADVIA 2120 Haematology System (Siemens Health Care Diagnostic Inc., Deerfield, USA). Differential leukocyte counts were carried out manually on Giemsa stained thin blood films. The values of the erythrogram and leukogram parameters were compared with the reference values as described by Meinkoth and Clinkebeard[8].

The data on ages, sexes, clinical manifestations and concomitant infections were obtained from the clinical histories filled out during the consultation, clinical examination and parasitological results.

3. Results

With regard to age, it was found that 50.00% evaluated dogs were less than one-year-old, and the remainder was distributed in several age groups up to 14-year-old. Samples were collected from 55.88% males and 44.12% females. About 58.82% dogs were of non-descript breed and 41.17% were of six different breeds. Of the 34 dogs for whom peripheral blood films were evaluated, 88.23% presented parasitaemia only of *H. canis* (Figure 1), while 11.77% had combination of *H. canis*, *Babesia* sp. and *Ehrlichia* sp. However, the parasitaemia was low in 88.23% of the cases (≤3%).

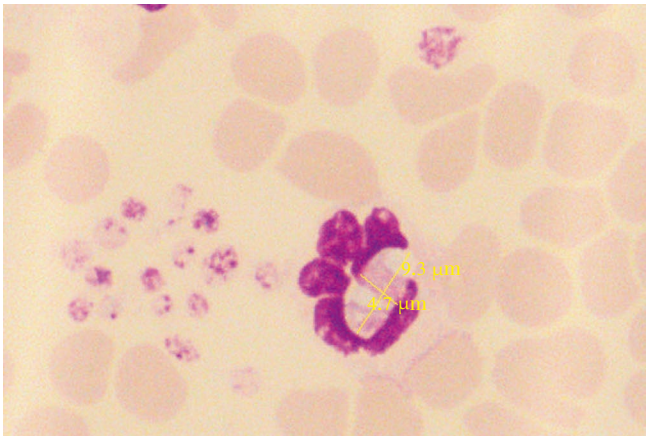

Figure 1. Microphotographs of *H. canis* during micrometric measurements by software DP2-BSW (OLYMPUS) in Wright Giemsa stained blood smear.

Anorexia, mild to moderate fever, pale mucosae, weight loss and lethargy were common clinical symptoms; a few were also showing signs of vomiting and diarrhea (Table 1).

Table 1
Clinical signs observed in the dogs naturally infected by *H. canis* (*n*=34).

Symptoms	Animals	Percentage (%)
Anorexia/inappetence[a]	9	26.47
Anorexia/inappetence[b]	15	44.11
Fever (mild to moderate)	21	61.76
Diarrhea	6	17.64
Vomiting	8	23.53
Lethargy	16	47.06
Pale mucosae	19	55.88
Weight loss	7	20.59
Dehydration of varied degrees	6	17.64
Corneal opacity	1	2.94
Enlarged lymphonodes	1	2.94

[a]: As only sign; [b]: In association with other signs.

In this study, the values of the erythrogram and leukogram parameters of the animals varied widely. The mean erythrocyte, haemoglobin and hematocrit values were lower than the reference values described by Meinkoth and Clinkebeard[8], while the leukocytes were higher than the reference range (Table 2). The haematological alterations mainly consisted of anaemia (73.53%), leukocytosis (44.11%),

Table 2
Values of the haematological parameters of 34 domestic dogs infected naturally by *H. canis*.

Parameters	Mean±SD	Minimum	Maximum	Reference
RBC ($\times 10^6$ cells/μL)	4.48±1.28	1.81	8.14	5.50-8.50
Hemoglobin (g/dL)	9.40±2.89	4.70	14.00	12.00-18.00
Hematocrit (%)	28.45±8.71	14.00	42.00	37.00-55.00
WBC (cells/μL)	17 071.80±7 078.90	5 100.00	27 790.00	6 000-17 000
Total neutrophils (cells/μL)	13 637.70±2 240.80	3 570.00	23 343.00	3 000-11 800
Lymphocytes (cells/μL)	3 067.90±2 408.40	239.00	7 026.00	1 000-4 800
Eosinophils (cells/μL)	366.30±405.20	0.00	1 263.00	100-1 250

RBC: red blood cells; WBC: white blood cells; SD: standard deviation.

neutrophilia (44.11%) and eosinopenia (35.29%).

4. Discussion

Canine hepatozoonosis is a disease which is presently prevalent in the various regions of India. *H. canis* is the species recognized as causing the infection[4-6], which prevails in dogs of all ages. In this study, the most frequent symptoms and haematological alterations were observed. The results indicate that *H. canis* is responsible for causing pathogenicity even in low infections. The presence of the parasite, especially in dogs up to the age of one year, corroborates the statement that animals of all ages can become infected, although it is more prevalent among younger dogs and this prevalence may be ascribed to the low immunity of young animals[9]. Infection by this haemogregarian haemoprotozoan in a significantly higher proportion of male dogs is in agreement with the findings of various workers[9,10], who reported a higher prevalence of infection among males. However, Jittapalapong *et al.* reported that there was no predisposition of sex for this infection[11]. The higher occurrence of males in this study might be due to the predominance of male dogs in the group under study.

In this study, the larger proportion of parasitism among non-descript dogs was due to their higher proportion among the dogs attended at the clinics. Hepatozoonosis does not show a predisposition for any particular breed.

In this study, four dogs had concomitant infections, and *Ehrlichia* was the agent most frequently found in association with *H. canis* (75%). The association of *H. canis* with other hematozoa can be attributed to the presence of the common tick vector, *Rhipicephalus sanguineus*, which is also a transmitter of *Ehrlichia canis* and *Babesia gibsoni*. Studies on tick biology indicate that a small percentage of ticks are responsible for harbouring multiple pathogens and successfully transmitting all pathogens to host or at some times[12]. Further more, the possible extant of co-infection could be due to myeloperoxidase deficiency in *H. canis* infected neutrophils[12].

The variations observed in the haemogram and leukogram parameters of the dogs may be related to parasitaemia, response of each animal and concomitant infection by other infectious agents.

The anaemia observed in 73.53% dogs in this study may be associated with the chronicity of the infection, as well as with concomitant infection by *Ehrlichia canis* and/or *Babesia* sp. For some researchers, anaemia is the commonest, primary haematological sign observed in most cases[7,13]. In the present study, normocytic normochromic anaemia was mainly found. Neutrophilia and leukocytosis in the animals of this study are consistent with the findings of other researchers, who stated these alterations are frequent[9,14]. However, in some studies, neutropenia has also been found[15]. These changes might be due to the multiplication of the parasite in animal's organs, hence resulting in inflammatory response which might be further increased in presence of other haemoprotozoa or bacterial infection. It has been found that concurrent infections with pathogens such as *Babesia canis* and *Ehrlichia* spp. aggravate the clinical condition of dogs infected with *H. canis*[16].

These clinical and hematological findings, though not specific for canine hepatozoonosis, reinforce the hypothesis that the presence of this agent in dogs should be taken into account even when parasitaemia is low.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Hepatozoonosis, an enzootic haemoprotozoan tick borne disease, is caused by *H. canis* in dogs. The disease occurs in mild to severe forms with variable signs including fever, paralysis, anorexia, emaciation, anaemia, ocular discharge and hindleg weakness. The authors have studied clinical and hematological aspects of dogs naturally infected with *H. canis*.

Research frontiers

Clinical signs in 34 dogs naturally infected by *H. canis* were observed, both in a single infection and in association with other haematozoa. Retrospectively description of the clinical and haematological alteration in dogs was made, and a beautiful microphotograph of *H. canis* was showed intuitively.

Related reports

This report described varied clinical signs in *H. canis* infection of dogs. Data about *Hepatozoon* and *Trypanosoma* mixed infection in amphibians were showed a highly infection and results in the decline of the size of population. It also showed a haematological alteration and immune system change.

Innovations and breakthroughs

The report described a good number of cases including concurrent infections with *H. canis*. The details include descriptive clinical signs. Low parasitaemia has been reported in 88.23% of the cases. About 25% of cases did not show any clinical signs except anorexia.

Applications

H. canis causes clinical and hematological alterations of varied degrees in dogs even with low parasitemia. It is concluded that even with low *H. canis* may cause clinical and haematological alteration in dogs. We may considerate the treatment against *H. canis* on pets and pay more attention to such imformation about hepatozoonosis in dogs.

Peer review

This report is a good retrospective study in a large number of cases describing clinical and hematological findings which is of use for clinicians in diagnosis of the condition. The data are interesting and enlightening other researchers focus on further investigations.

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