



First case report of canine infection with *Hepatozoon canis* (Apicomplexa: Haemogregarinidae) in Himachal Pradesh

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Abstract

This report describes the detection and effective management of *Hepatozoon canis* infection in a male dog for the first time from Himachal Pradesh. The anamnestic information of affected dog revealed anorexia, shivering, weakness, vomition, lethargy and un-responsiveness to antibiotic therapy. Clinical examination unveiled fever, pale mucous membranes, enlargement of prescapular lymph nodes and dullness. Clinical findings, hemato-biochemical parameters, parasitological and therapeutic management strategies were then evaluated. Important hematological outcomes consisted of anemia, thrombocytopenia, and leukocytosis. Parasitological examinations revealed gamonts of *H. canis* within neutrophils in thin blood smears. The dog was successfully treated using combination drug therapy consisting of imidocarb dipropionate and doxycycline, which had proven therapeutic efficacy against hepatozoonosis.

Key words: First report, *Hepatozoon canis*, Dog, Himachal Pradesh.

Canine hepatozoonosis caused by *Hepatozoon canis* (Apicomplexa, Eucoccidiorida, Hepatozoidae), is an important vector-borne disease of dogs with worldwide distribution. The causative agent has a complex and unique life cycle, with ticks and canines acting as definitive and intermediate hosts, respectively (Smith 1996; Ivanov and Tsachev 2008). The brown dog tick, *Rhipicephalus sanguineus* (Ixodida: Ixodidae), is the principal vector responsible for occurrence of canine hepatozoonosis (Baneth *et al.* 2001, Singh *et al.* 2017). Dogs become infected by ingesting tick harbouring sporulated oocysts of the parasite. After ingestion, sporozoites are released and penetrate the intestinal epithelium, where they disseminate to the hemolymphatic tissues via lymphatics or blood vessels of infected dog. Sporozoites undergo merogony in the bone marrow, spleen, lymph nodes and other tissues. Merozoites are released and invade leukocytes (neutrophils and monocytes) forming gamonts, which are then ingested

by ticks, undergo a sexual stage, and where the oocysts are formed (Baneth *et al.* 2007).

Depending on the level of parasitaemia and immune response of the infected host, the infections may vary from asymptomatic to mild or severe and potentially fatal (Klopfer *et al.* 1973). The severe form of disease is typically manifested by fever, anorexia, lethargy, weight loss, lymphadenomegaly and anaemia (Chhabra *et al.* 2013). The infection is frequently diagnosed in stained peripheral blood smears by microscopic detection of ellipsoidal shaped intracellular gamonts within neutrophils or monocytes, and visualization of “wheel-spoke” meronts or monozytic cysts in tissues in histopathological specimens (Baneth and Shkap 2003)

There are number of reports suggesting the global occurrence of *H. canis* infection among dogs (Murata *et al.* 1993; Baneth and Weiger 1997; Allen *et al.* 2008; Sakuma *et al.* 2009; Otranto *et al.* 2011). Likewise,

disease has also been reported from various states of India (Ingole *et al.* 2011; Rani *et al.* 2011; Pawar *et al.* 2012) including a few from neighboring state of Punjab (Chhabra *et al.* 2013; Singh *et al.* 2017). However, hepatozoonosis in dogs has never been reported from the north western Himalayan region of India. Therefore, to the best of our knowledge, this paper describes the clinical report and subsequently the successful management of canine hepatozoonosis for the first time from the state of Himachal Pradesh.

Materials and Methods

A nine year old non-descript male dog was presented to outpatient unit in the department of Veterinary Clinical Medicine Dr. G. C. Negi College of Veterinary and Animal Sciences, Palampur (H.P.) with anorexia, shivering, weakness, vomiting, lethargy and un-responsiveness to antibiotic therapy. Clinical examination further revealed elevated body temperature (101°F), pale mucous membranes and enlarged prescapular lymph nodes.

The collected blood samples were immediately analyzed for determination of haemoglobin concentration [Hb (g%)]; packed cell volume [PCV (%)]; total white blood cell count [TLC ($\times 10^3/\mu\text{L}$)] and total platelet count [PLT ($\times 10^3/\mu\text{L}$)] using fully automated blood analyzer, ADVIA 2120 Haematology System (Haematology Analyzer BC 2800 Vet, Fresenius medical care Ltd). The Differential leucocyte count was performed manually under oil immersion power (100X) of bright field microscope in Giemsa stained thin blood smear to count 100 leucocytes per slide. For Biochemical profile, the serum samples were separated from whole blood samples and the biochemical profile was determined according to the standard methodology, using an automated biochemistry analyzer (Microlab 300 clinical Chemistry, Merck Ltd. Mumbai) with commercially available reagent kits. The biochemistry panel included total protein (TP), blood urea nitrogen (BUN), alkaline phosphatase/ALP, alanine aminotransferase/ALT, aspartate transaminase/AST, bilirubin, glucose and creatinine. For the parasitological diagnosis of suspected haemoprotozoa, the thin blood smears prepared by Giemsa staining method were microscopically examined (Soulsby 1982). Further, radiographic examination was done using Siemens 80 MA mobile X-ray machine (Siemens Ltd, Goa) to rule out any

visceral abnormality.

Results and Discussion

This report describes a 9 year old non-descript male dog, from Kangra, Himachal Pradesh, infected with *Hepatozoon canis*. The definite diagnosis was reached based on clinical signs and laboratory findings. A complete blood count (CBC) indicated leukocytosis, moderate anemia and mild thrombocytopenia (Table 1). The biochemical abnormalities included increase in alkaline phosphatase, alanine aminotransferase/ALT, aspartate transaminase, bilirubin, blood urea nitrogen, creatinine, glucose and total protein (Table 1). Leucocytosis with shift to left was also observed (Neutrophil 86.5%, Monocyte 3%, Lymphocytes 10.5%). Radiological examination revealed splenomegaly which could possibly be attributed to reactive lymphoid hyperplasia and concurrent extramedullary hematopoiesis (Egenvall *et al.* 2000). The results obtained were tentatively indicating *H. canis* infection which was in agreement with the earlier reports of various researchers on clinical canine hepatozoonosis (Chhabra *et al.* 2013; Pais *et al.* 2016; Singh *et al.* 2017). On blood smear examination, gamonts of *H. canis* were finally observed in neutrophils and infection was confirmed as canine hepatozoonosis (Fig 1). Parasitaemia varied from 6 – 35% of the circulating neutrophils.

Based on these observations, the present case was diagnosed as canine hepatozoonosis. Such infections with *H. canis* has been recognized in dogs from the regions of Asia (Murata *et al.* 1993, Sakuma *et al.* 2009), Mediterranean basin (Sasanelli *et al.* 2010), Middle East (Baneth and Weiger 1997), South America (Eiras *et al.* 2007), Southern states of the USA (Allen *et al.* 2008) and Europe (Ontranto *et al.* 2012). The infections have also been reported from parts of Indian subcontinent (Pawar *et al.* 2012, Rani *et al.* 2011, Ingole *et al.* 2011), wherein the brown dog ticks, *Rhipicephalus sanguineus* were noticed on the skin surface (Fig 2). While *R. sanguineus* is considered as principal vector of *H. canis*, tick species such as *Amblyomma ovale* have also been shown to be the alternative or potential vectors (Rubini *et al.*, 2009).

Combined drug therapy with doxycycline @ 6 mg/kg body weight orally for 21 days and Inj. imidocarb dipropionate @ 6 mg/kg body weight

Table 1. Values of the haemato-biochemical parameters observed in a dog infected with *Hepatozoon canis*

| Parameters | *Reference values | Observed values |
|-------------------------------------|-------------------|-----------------|
| Hb (g%) | 12-18 | 10.8 |
| PCV (%) | 37-55 | 32.4 |
| TLC ($\times 10^3 / \mu\text{L}$) | 6-17 | 21.3 |
| PLT ($\times 10^3 / \mu\text{L}$) | 200–500 | 630 |
| ALP U/ L | 20-156 | 170 |
| ALT U/ L | 4.8-24 | 28 |
| AST U/ L | 10-40 | 212 |
| Bilirubin mg% | 0.1-0.3 | 0.07 |
| BUN % | 4-27 | 26.1 |
| Creatinine mg | 0.5-1.5 | 2.9 |
| Glucose mg% | 80-150 | 200 |
| Total protein | 5.4-7. | 17.6 |

* Reference values according to Rizzi *et al.* (2010)

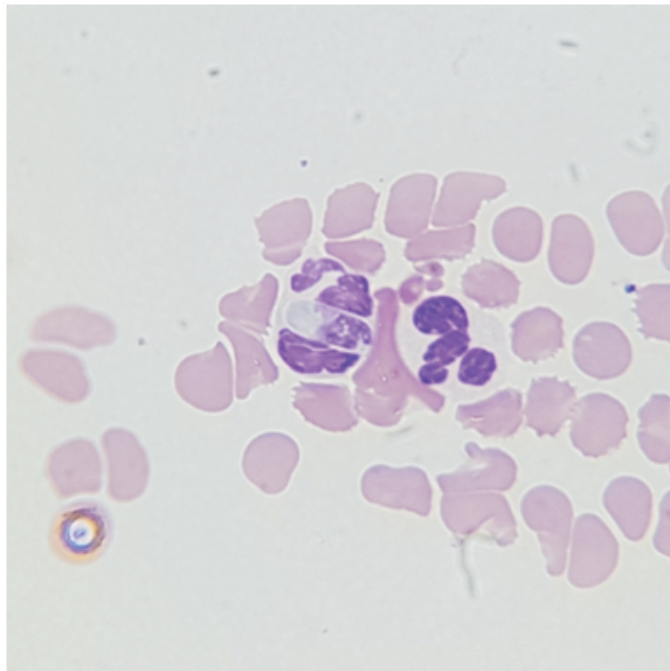


Fig.1 *Hepatozoon canis* gamont



Fig 2. *Rhipicephalus sanguineus* spp.

subcutaneously and repeated after 14 days, exhibited depression of gametocyte parasitaemia accompanied by clinical recovery of the presented dog. These two drugs i.e. imidocarb dipropionate and doxycycline has previously been reported as the drugs of choice for treatment of *H. canis* infection (Lappin 2010; Kwon *et al.* 2017) and the prognosis has been reported as good (Ibrahim *et al.* 1989). Therefore, the present findings were in concordance with the earlier reports. Although, there are certain reports of failure of imidocarb therapy in dogs but its combination with doxycycline showed a good response in the present study which is in agreement with the findings of Sasanelli *et al.* (2010).

In conclusion, this is for the first time, *H. canis*

infection has been reported, documented and efficiently managed in a dog from Himachal Pradesh, a state in the north western Himalayan region of India. With the established facts such as prevalence of *H. canis infection* in adjoining state of Punjab and the other contributing factors like increased mobility and purchase of pets from kennels in Punjab, the occurrence of tick vector species in the study area etc., there is very high probability that the pets in Himachal Pradesh may acquire *H. canis infection*, if the situation is ignored or overlooked. Hence, in view to prevent occurrence and for the successful management of the existing disease, early diagnosis on the basis of clinical findings and laboratory investigations assumes utmost importance.

References

- Allen KE, Li Y, Kaltenboeck B, Johnson EM, Reichard MV, Panciera RJ and Little SE. 2008. Diversity of *Hepatozoon species* in naturally infected dogs in the southern United States. *Veterinary Parasitology* **154**:220-225.
- Baneth G and Shkap V. 2003. Monozoic cysts of *Hepatozoon canis*. *Journal of Parasitology* **89**:379–381.
- Baneth G and Weigler B. 1997. Retrospective Case-Control Study of Hepatozoonosis in Dogs in Israel. *Journal of Veterinary Internal Medicine* **11**: 365-370.
- Baneth G, Samish M and Shkap V. 2007. Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*) *Journal of Parasitology* **93**:283–299.

- Baneth G, Samish M, Alekseev E, Aroch I and Shkap V.2001. Transmission of *Hepatozoon canis* to dogs by naturally-fed or percutaneously-injected *Rhipicephalus sanguineus* ticks. *Journal of Parasitology* **87**:606–611.
- Chhabra S, Uppal SK, Singla Lachhman Das and Zhou Huaiyu. 2013. Retrospective study of clinical and hematological aspects associated with dogs naturally infected by *Hepatozoon canis* in Ludhiana, Punjab, India. *Asian Pacific Journal of Tropical Biomedicine* **3**:483-6.
- Egenvall A, Lilliehook I, Bjoersdorff A, Engvall EO, Karlstam E, Artursson K, Heldtander M and Gunnarsson A. 2000. Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs. *Veterinary Record* **146**:186–190.
- Basabe J, Scodellaro CF, Banach DB, Matos ML, Krimer A and Baneth G. 2007. First molecular characterization of canine hepatozoonosis in Argentina: evaluation of asymptomatic *Hepatozoon canis* infection in dogs from Buenos Aires. *Veterinary Parasitology* **149**:275-279.
- Ibrahim ND, Rahamathulla PM and Njoku CO. 1989. Neutrophil myeloperoxidase deficiency associated with canine hepatozoonosis. *International Journal for Parasitology* **19**:915-918.
- Ingole KH, Sawale Ganesh, Rohi RR, Suryavanshi PR, Sabale SS, Bharkad GP and Vishwasrao SV. 2011. Clinico-pathology and therapeutic management of hepatozoonosis in dogs: Case study. *Journal of Veterinary Parasitology* **25**:159-161.
- Ivanov A and Tsachev I. 2008. Hepatozoon canis and hepatozoonosis in the dog. *Trakia Journal of Sciences* **6**(2):24.
- Klopfer U, Neuman F and Nobel TA.1973. *Hepatozoon canis* infection in dogs in Israel. *Refu Veterinary* **1**(30):116–120.
- Kwon SJ, Kim YH, Oh HH and Choi US.2017. First Case of Canine Infection with *Hepatozoon canis* (Apicomplexa: Haemogregarinidae) in the Republic of Korea. *Korean Journal of Parasitology* **55**(5):561-564.
- Lappin MR.2010. Update on the diagnosis and management of *Hepatozoon spp* infections in dogs in the United States. *Topics in Companion Animal Medicine* **25**: 142-4(2010).
- Murata T, Inoue M, Tateyama S, Taura Y and Nakama S.1993. Vertical transmission of *Hepatozoon canis* in dogs. *The Journal of Veterinary Medical Science, the Japanese Society of Veterinary Science* **55**: 867-868.
- Otranto D, Dantas-Torres F, Weigl S, Latrofa M S, Stanneck D, Decaprarriis D, Capelli G and Baneth G. 2011. Diagnosis of *Hepatozoon canis* in young dogs by cytology and PCR. *Parasites and Vectors* **4**: 55.
- Paiz LM, Silva RC, Satake F and Fraga TL. 2016. Hematological disorders detected in dogs infected by *Hepatozoon canis* in a municipality in Mato Grosso do Sul State, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **68** (5):1187-1194.
- Pawar RM, Poornachandar A, Srinivas P, Rao KR, Lakshmikantan U and Shivaji S. 2012. Molecular characterization of *Hepatozoon spp.* infection in endangered Indian wild felids and canids. *Veterinary Parasitology* **186** (3-4):475-9.
- Rani PAMA, Irwin PJ , Coleman G T , Gatne M and Traub R J . 2011. A survey of canine tick-borne diseases in India. *Parasites & Vectors* **4**:141.
- Rizzi TE, Meinkoth JH and Clinkenbeard KD. 2010 Normal hematology of the dog. In: WEISS DJ, WARDROP KJ (Eds.). *Schalm's veterinary hematology*. 6thed. Ames: Wiley-blackwell, 2010. pp. 799-810.
- Rubini AS, Paduan KS, Martins TF, Labruna MB and O'Dwyer LH. 2009. Acquisition and transmission of *Hepatozoon canis* (Apicomplexa: Hepatozoidae) by the tick *Amblyomma ovale* (Acari: Ixodidae). *Veterinary Parasitology* **164**:324-327.
- Sakuma M, Nakahara Y, Suzuki H, Uchimura M, Sekiya Z, Setoguchi A and Endo Y. 2009. A case report: a dog with acute onset of *Hepatozoon canis* infection. *The Journal of Veterinary Medical Science the Japanese Society of Veterinary Science* **71**:835-838.
- Sasanelli M, Paradies P, Greco B, Eyal O, Zaza V and Baneth G. 2010. Failure of imidocarb dipropionate to eliminate *Hepatozoon canis* in naturally infected dogs based on parasitological and molecular evaluation methods. *Veterinary Parasitology* **171**:194-199.
- Singh K, Singh H, Kumar NK, Kashyap N, Sood N and Rath SS. 2017. Molecular prevalence, risk factors assessment and haemato-biochemical alterations in hepatozoonosis in dogs from Punjab, India. *Comparative Immunology, Microbiology and Infectious Diseases* **55**: 10.1016/j.cimid.2017.10.001.
- Smith TG. 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). *Journal of Parasitology* **82**:565–585.
- Soulsby E.J.L. 1982. *Hepatozoon canis* in Helminths, Arthropods and Protozoa of Domesticated Animals. 7th Edn. ELBS and Bailliere Tindall, London pp. 689-691.