Molecular Diagnosis and Therapeutic Management of *Hepatozoon canis* Infection in Dogs

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ABSTRACT

The present paper describes the diagnosis and therapeutic management of Hepatozoon canis in dogs. Two male Labrador dogs of 1 year age with history of weakness, lethargy and inappetance were presented to University Veterinary Hospital. Clinical examination revealed pale mucous membranes, enlargement of lymph nodes, weakness, lethargy and infestation of ticks (Rhipicephalus sanguineus). The blood smears examination showed typical gelatin capsule shaped gamonts within the neutrophils suggestive of H. canis in one dog, whereas, other dog was found negative. Low haematocrit values and thrombocytopaenia were recorded in haematology. The species specific polymerase chain reaction assay targeting 18SrRNA gene yielded amplicons of 666 bp suggestive of H. canis from the DNA from the blood of both the dogs. Even though H. canis infection is generally in apparent in dogs, severe disease can occur in immunosuppressed dogs or those with concurrent infections. So control of vectors has to be considered as an important measure to reduce this vector borne disease.

Keywords*: Hepatozoon canis,* canine, PCR, Kerala

INTRODUCTION

Canine hepatozoonosis, tick borne hemoparasitic diseases, is caused by *Hepatozoon canis* and transmitted by infected tick vectors. *H. canis* infection ranges from a subclinical state with low level parasitaemia, to a severe lifethreatening illness with fever, lethargy, anaemia and emaciation in dogs with high parasitaemia (Banethand Weigler, 1997). Immunosuppression caused by concurrent diseases or other factors appears to play an important role in the manifestation of significant clinical signs. If parasitemia is distinct, diagnosis of infection can readily be confirmed by demonstration of typical intracytoplasmic ellipsoidal-shaped gamonts within the neutrophils in blood smears (Ibrahim *et al.,* 1989). Blood smear examination fails to demonstrate the parasite when parasitaemia is low or intermittent (Baneth and Shkap, 2003). Hence, the recent diagnostic techniques such as enzyme-linked immunosorbent assay, indirect fluorescent antibody test and PCR are employed also detect the low degree of *Hepatozoon* infection (Gonen *et al*., 2004; Li *et al*., 2008). Molecular method like PCR are considered as the most useful tool for diagnosis of subclinical and latent infections when the parasitaemia is low. The present paper discusses on molecular diagnosis and therapeutic management of *H. canis* infection in dogs.

MATERIALS AND METHODS

Two Labrador dogs of 1-year age belonging to police academy were presented to University Hospital, with history of weakness, lethargy and inappetance. After through clinical examination, blood samples were collected from peripheral veins of ear tip and subjected to Wet film examination and microscopical examination of blood smears after staining with Giemsa stain. The whole blood samples were also collected and genomic deoxyribonucleic acid (DNA) was extracted using commercial DNA extraction kits. The extracted DNA was used for species specific PCR for *H.canis* targeting 18SrRNA gene using primers, Hep F 5'-ATACATGAGCAAAATCTCAAC-3' and HepR 5'CTTATTATTCCATGCTGCAG-3'. The composition of PCR mix (25.0 µL) included 12.5 µl master mix, 1.5µL of 10 pmol primers, 1 µl of template DNA and 3.5 µl nuclease free water. The PCR programme was carried out in a thermocycler under the following conditions; initial denaturation at 95°C for 5 minutes, 35 cycles of amplification with denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds and extension at 70°C for 1.30 minutes and final extension at 72°C for 5 min. The amplicons were visualized by gel electrophoresis on 1.0 per cent agarose and documented. The PCR products were sequenced and subjected to blast analysis.

RESULTS AND DISCUSSION

On basis of clinical examination and results of blood smear and PCR using species specific PCR for *H.canis*, the dogs were diagnosed with *H. canis* infection (Figs. 1 & 2). The haematology revealed anaemia and thrombocytopaenia and the findings are in accordance with earlier investigations (Inokuma *et al.*, 2002; Chhabra *et al.*, 2013). Anemia was reported to be the most frequent hematological alteration associated with hepatozoonosis (Paiz *et al*., 2016). In this study, the PCR products were sequenced and the nucleotide sequence was submitted to Genbank and allotted accession number MF797806. Earlier, Singla *et al.* (2017) diagnosed *H. canis* infection in dogs by PCR in Panjab and sequence analysis of 18SrRNA gene revealed that *H. canis* detected in north Indian dogs might have closer ancestral relationship with Saint kitts and Nevis strain. The PCR assay is reported to detect parasite even in low parasitaemia because of its high sensitivity (Attipa *et al*., 2018).

The animals were successfully treated withsingle dose of Imidocarb dipropionate @ 6 mg/ kg BW followed by parenteral administration of

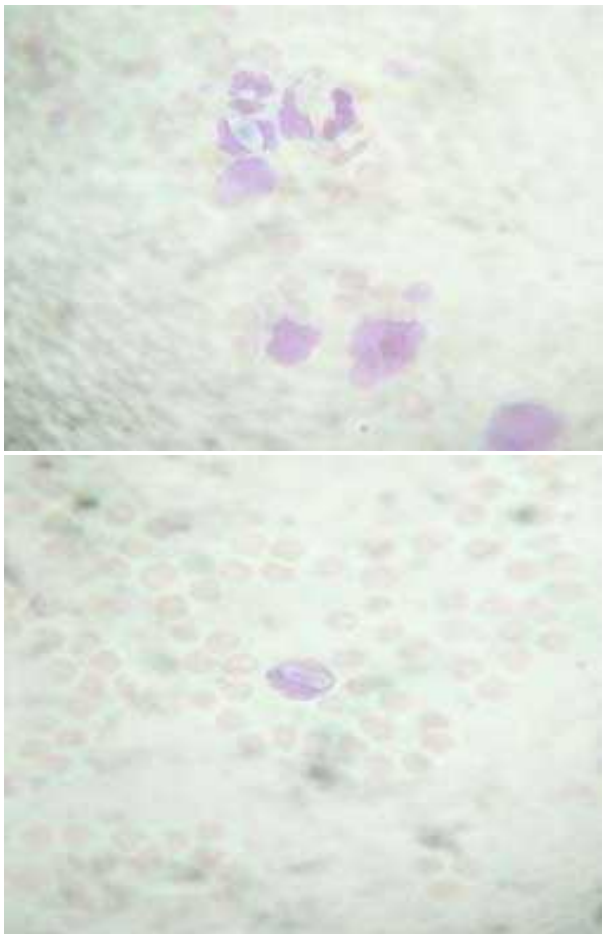

Fig. 1: Plate 1&2 gelatin capsule shaped gamont within the neutrophils

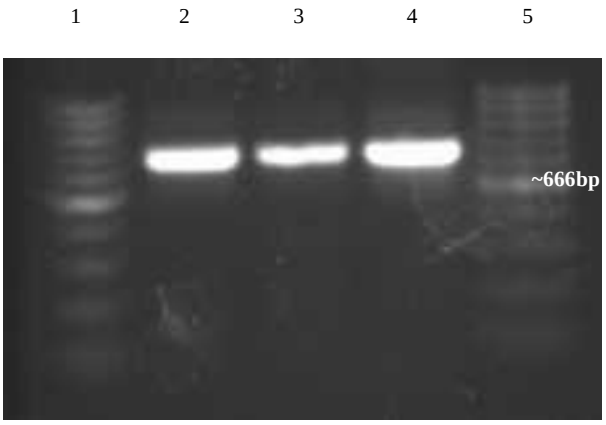

Fig. 2: PCR products of *H.canis*
Lane 1 & 5: 100bp DNA ladder
Lane 2: Positive control
Lane 3 & 4: Positive test samples

sulpha trimethoprim @ 15 mg/kg BW for five days along with haematinics and vitamin supplements as supportive therapy. Blood smear examination revealed no parasites after the course of treatment. No single drug was found to be effective for complete elimination of the organisms. Hence, combination of two or more drugs including imidocarb dipropionate, tetracycline, doxycycline, sulpha-trimethoprim, clindamycin or pyremethamine may be effective (Macintire *et al*., 2001). Sarma *et al*. (2012) reported successful treatment of *H. canis* infected dogs with combination therapy using doxycycline and oxytetracycline. Even though imidocarb dipropionate was considered as the specific drug for hepatozoonosis, it fails to eliminate *H.canis* when used as a sole therapy (Baneth, 2011). Prognosis often depends on the degree of parasitaemia and dogs with low parasitaemia typically respond well to treatment. But response to treatment may be poor in those with high parasitaemia and with concurrent illness.

In conclusion, the PCR assay is superior over blood smear examination for diagnosis of *Hepatozoon canis* infection in low parasitaemia dogs. Although *H. canis* infection is generally inapparent or subclinical in nature in dogs, the disease is manifested as severe form in immunocompromised dogs with concurrent infections. Therefore, control of vectors has to be considered as an important measure to reduce this vector borne disease. A further detailed study is warranted to assess the status of hepatozoonosis among canine population of Kerala.

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