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Molecular detection of *Hepatozoon* spp. in domestic cats of Kerala#

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Abstract

Vector-borne diseases are an important cause of morbidity and mortality in domestic cat population and hepatozoonosis is one such infection. Incidence of hepatozoonosis has been described in a variety of animal species but information on cats is scarce. To investigate the occurrence of this pathogen in domestic cats, blood samples were collected from domestic cats from three districts of Kerala (Thrissur, Kannur and Wayanad). Field-stained blood smears were examined to detect the presence of gamonts of Hepatozoon spp. and the samples were subjected to molecular analysis by PCR amplification. Out of 122 blood samples screened, none of the sample revealed the presence of gamonts of Hepatozoon on microscopic examination. Polymerase chain reaction targeting 18S rRNA gene of Hepatozoon spp. demonstrated seven positive cases with a prevalence of 5.74 per cent. Results of this study indicate that hepatozoonosis is established within the domestic cats in Kerala and warrant the adoption of control measures.

Keywords: *Vector-borne diseases, hepatozoonosis, molecular analysis*

The genus *Hepatozoon* includes Apicomplexan parasites of the family *Hepatozoidae*. Currently more than 340 species of *Hepatozoon* affecting mammals, birds, reptiles and amphibians have been identified. The first report of hepatozoonosis in domestic cat was from India in 1908. Several countries from South Africa, Asia, southern Europe, South America and USA have reported the infection in domestic cats. Three different *Hepatozoon* species were described to infect domestic cats which include, *H. felis, H. canis* and *H. silvestris*. Among them *H. felis* has been the most frequently diagnosed species in cases of feline hepatozoonosis in different countries around the world (Basso *et al.,* 2019).

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The gamont stage of *Hepatozoon* spp. circulates in leukocytes of infecting mammals and birds. The meronts are associated with muscle tissues especially, myocardium and skeletal muscles of domestic cats (Baneth, 2011). The infection does not lead to significant inflammatory reaction around the parasite meronts, so cats rarely develop clinical signs (Baneth *et al*., 2013). In domestic cats the infection is reported to be subclinical (Diaz-Reganon *et al.,* 2017). However, in stressed, immunocompromised cats or in concurrent infections, hepatozoonosis can be pathogenic.

Diagnosis is usually based on the demonstration of gamonts in the cytoplasm of neutrophils and monocytes in blood smears. But microscopy of blood smears is not sensitive enough for the detection of *Hepatozoon* gamonts, due to the limited level of parasitaemia, with less than one per cent of neutrophils and monocytes containing gamonts (Pereira *et al.,* 2019). Polymerase chain reaction is more sensitive than blood smears examination for diagnosis of Hepatozoon infection (Baneth *et al.,* 2013). The present study has been carried out to investigate the occurrence of hepatozoonosis among domestic cats of Kerala.

Materials and methods

A total of 122 domestic cats (from Thrissur, Wayanad and Kannur districts of Kerala, India) were screened for hepatozoonosis. Blood smears and whole blood in EDTA vial were collected from the animals. Blood smears were stained with Field stain and examined microscopically for the detection of *Hepatozoon* spp. gamonts (1000X magnification). DNA was extracted from whole blood using a commercial extraction kit (Qiagen, DNeasy Blood & Tissue Kit, Germany) following the manufacturer's instructions. Polymerase chain reaction was performed using the 18SHepF/18SHepR primers (Inokuma *et al.*, 2002) targeting a partial sequence of the 18S rRNA gene of *Hepatozoon* spp. (Table 1). Conventional PCR was performed in total volume of 25µl consisting of PCR master mix (Sapphire, Takara) (12.5µl), nuclease-free water (7.5µl), 1µl of each primer and 3µl DNA template. Amplification was performed using a programmable conventional thermocycler (MJ Mini™ Personal Thermal cycler, Bio-Rad). Initial denaturation at 95°C for 5 min, was followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec and final extension at 72°C for 90 sec. After the last cycle, the extension step was continued for a further 5 min. The DNA extracted from blood of dog infected with *H. canis* was included as positive control. The amplicons were electrophoresed on 1.2 per cent agarose gels and evaluated.

Results and discussion

Microscopical examination of Field-stained blood smears from 122 cats could not reveal gamonts of *Hepatozoon* spp. Polymerase chain reaction targeting 18S rRNA gene of *Hepatozoon* spp. demonstrated seven positive cases with a prevalence of 5.74 per cent (Fig. 1). Sequencing of one sample which yielded amplicon of approximately 666 bp of *Hepatozoon* spp. was carried out and the sequence submitted to NCBI Genbank (Accession No: OP257232). The isolate showed 99.37 per cent identity with *H. felis* isolate from wild (Jungle) cat collected from Wayanad, Kerala and 99.05 per cent similarity towards *H. felis* isolate of a tiger from Trivandrum Zoo.

Pereira *et al.* (2019) and Grillini *et al.* (2021) also reported absence of gamonts in peripheral blood smears in studies conducted in Maio Island, Republic of Cape Verde and North-Eastern Italy respectively. However, Jittapalapong *et al.* (2006) and Morelli *et al.* (2021) detected gamonts of *Hepatozoon* spp. in the study population in Thailand and Greece respectively. Giannelli *et al.* (2017) detected 5.1 per cent occurrence of feline hepatozoonosis

Table 1. Primer sequences targeting 18S rRNA gene specific for *Hepatozoon* spp.

Name of gene	Primer sequences	Amplicon size
18SHepF	5 ′ATACATGA GCAAAAT CTCAAC 3′	~666bp
18SHepR	5 ′CTTATT ATTCCATG CTGCAG 3′	

in a molecular study conducted in southern Italy. Lower prevalence was reported by Diaz-Reganon *et al.* (2017) from Madrid, Spain and Oliveira *et al.* (2018) from domestic cats in Angola. Higher prevalence was reported from Kerala by Malangmei *et al.* (2021), Morelli *et al.* (2021) in Greece and Attipa *et al.* (2017) in cats of Cyprus.

Among the positive samples, four cats were within the age group of one to two years (57.14 per cent), two were 6-12 months of age and one was below six months. Similar finding was reported by Baneth *et al.* (1998). But Maia *et al.* (2014) reported that the prevalence of *Hepatozoon* spp. was higher in cats older than 60 months (5 years).

According to the present study, almost equal distribution was noticed among Domestic Short Hair (57.14 per cent) and Persian cats (42.86 per cent). This indicated absence of significance of breed in hepatozoonosis among domestic cats. But Baneth et al. (1998) and Schafer et al. (2021) reported higher prevalence of hepatozoonosis in local breeds. Among seven positive cats, five were males and two were females. Baneth et al. (1998) also reported higher incidence of hepatozoonosis among male cats which might be due to the roaming behaviour of male cats. However, Giannelli et al. (2017) and Pereira et al. (2019) reported that gender was not a risk factor for H. felis infection in cats. Season wise occurrence revealed equal distribution of cases during monsoon and post-monsoon and least prevalence in pre-monsoon. Baneth et al. (1998) and Diaz-Reganon et al. (2017) reported similar findings. It might be caused by a persistent or chronic infection among domestic cats.

Out of seven positive cats four were kept indoor, one outdoor and two were reared both indoor and outdoor. Grillini *et al.* (2021) also reported the high prevalence in indoor cats, that were commonly less exposed to vectors' activity due to their lifestyle, suggesting that alternative ways of transmission were possible (e.g., vertical transmission, predation of infected preys). Vilhena *et al.* (2013) reported that associations between housing conditions and prevalence of the infection were not found among cats in a study conducted in Portugal. With regard to the feeding habit five out of seven positive cats were fed with commercial diet and two with homemade diet. No reports were encountered mentioning the relationship of diet and occurrence of hepatozoonosis in domestic cats.

Clinical signs of *Hepatozoon* spp. infected cats included anorexia, sneezing, body wasting, urinary incontinence, diarrhoea and swaying back. Among these signs body wasting was observed in two cats. Ectoparasites were noted in three cats infected with *Hepatozoon* spp. Anaemia was evident in one case which was co-infected with *Babesia* spp. infection with *Babesia* spp. was associated with anaemic changes in dogs in Kerala (Anju *et al.,* 2022). Grilleni *et al*. (2021) reported that *Hepatozoon* infected cats were mostly sub-clinically infected, in apparently good physical condition and only in one case was diarrhoea and rhinitis were present.

Summary

In the present study, 5.7 per cent of the cats screened were positive for hepatozoonosis. PCR was found to be more sensitive than blood smears examination for diagnosis of feline hepatozoonosis. Based on the study, young male cats reared indoor without any preventive measures are more susceptible to the infection. Out of seven cats found positive for *Hepatozoon* spp. six were apparently healthy which indicates a sub-clinical infection or carrier status. Nevertheless, further studies are necessary to determine the real impact of this parasite in cats. Additional research is necessary, including a larger number of animals and feline populations from other districts, aiming at better characterising and controlling feline vector-borne pathogens and their arthropod vectors in Kerala.

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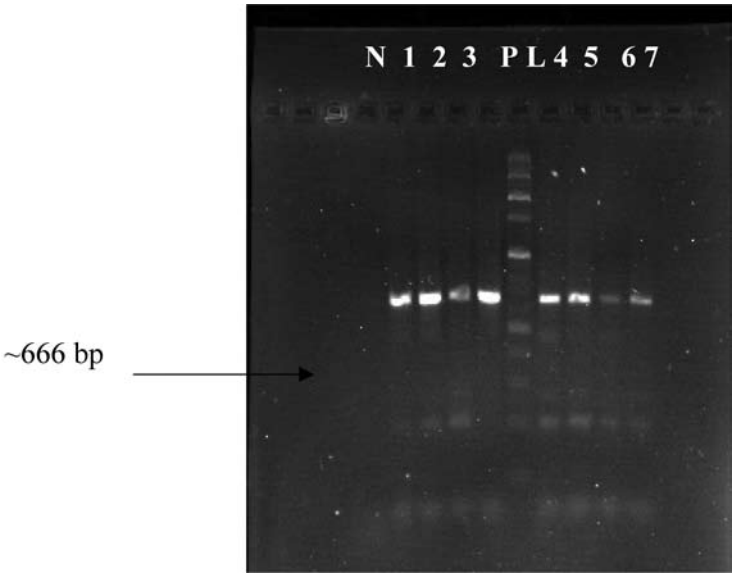

Fig. 1. Polymerase Chain Reaction amplification of 18S rRNA sequence of the *Hepatozoon* spp. from seven cats

N: Negative control, P: Positive control, L: 100bp Ladder, 1-7: Samples

Conflict of interest

The authors declare that they have no conflict of interest.

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