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Molecular identification of the *Hepatozoan canis* in *Rhipicephalus sanguineus* ticks in Tamil Nadu

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Abstract

This study investigated the presence of *Hepatozoan canis* in ixodid ticks *Rhipicephalus sanguineus sensu lato*, commonly referred to as the “kennel tick” or “brown dog tick.” Three hundred and twenty four semi engorged ticks were collected from a dog shelter and from naturally infected dogs from Chennai, Madurai and Namakkal districts in Tamil Nadu state, India to identify the *Hepatozoan canis* infections. The ticks were surface sterilized with ethanol and dissected to separate the midgut and ovary and the DNA was extracted, PCR amplification and sequencing was carried out to obtain a 666 bp fragment of the 3' half of the 18S rDNA sequences. The nucleotide sequences were compared with respective ones available in GenBank using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Sequence Tool (BLAST) search engine (<http://blast.ncbi.nlm.nih.gov/blast.cgi>). The nucleotide sequence of 18S rRNA gene of *H. canis* showed 98.10 percent homology with the sequences of other isolates of *H. canis* available in the gene bank. Among the 324 ticks examined 15, 25 and 20 per cent *H. canis* DNA was detected in pooled samples of *R. sanguineus* ticks collected from Chennai, Madurai and Namakkal respectively.

Keywords: *Hepatozoan canis*, PCR assay, midgut, Blast analysis

Introduction

Rhipicephalus sanguineus sensu lato ticks can transmit a variety of pathogens to dogs (Dantas-Torres *et al.*, 2012) [5], including bacteria such as *Ehrlichia canis*, *Rickettsia conorii*, *Rickettsia rickettsii* and protozoa (*Hepatozoan canis*, *Babesia vogeli*). *Rhipicephalus sanguineus s.l.* ticks are found worldwide, primarily in tropical and subtropical regions, due to their high adaptability to different environmental conditions (Dantas-Torres *et al.*, 2013; Giannelli *et al.*, 2013) [7]. Dogs, foxes and jackals can become infected by *H. canis* (Eucoccidiorida, Hepatozoidae) by ingestion of nymphs or adult ticks that harbour infective oocysts (Baneth, 2011; Giannelli *et al.*, 2013) [7]. *Hepatozoon canis* is a tick-borne apicomplexan parasite that causes a variety of symptoms in dogs from mild infection to severe illness characterized by cachexia, fever, weight loss and anaemia when parasitic infection is severe.

Canine hepatozoonosis has been reported from America, Africa, Asia, and Europe, with some areas (e.g., southern Italy) being particularly suitable for the occurrence of this infection (Baneth, 2011; Dantas-Torres *et al.*, 2012; Giannelli *et al.*, 2013) [2, 5, 7]. This study aimed to detect *H. canis* infection by molecular analysis in *R. sanguineus sensu lato* ticks collected from different places in Tamil Nadu state, India.

Materials and Methods

Tick collection and Morphological identification

A total of 324 semi engorged ticks from naturally infested dogs were collected from Blue Cross Society in Chennai, Veterinary Poly Clinic in Madurai, and Namakkal. From each location, a minimum of five semi-engorged ticks attached to dogs were collected. The collected ticks were identified morphologically and stored in 70% ethanol based on Barker and Walker (2014) [3].

Tick dissection

Each semi-engorged tick was externally sterilized for five to ten minutes in a 70% ethanol solution before being dissected, and then it was rinsed in sterilized distilled water. Every midgut dissection was carried out under a microscope. With a few minor adjustments, the ticks were dissected by removing the upper cuticle using a sterile blade in order to separate the midgut, following the procedure used by Lalzar *et al.* (2014) [10].

Extraction of genomic DNA

The genomic DNA extraction from dissected midgut using the DNeasy Blood and Tissue kit (Qiagen) following the instructions from the manufacturer. The concentration of genomic DNA was evaluated with a Nano Drop™ 2000 (Thermo Scientific, USA), and stored at -20 °C until further use.

PCR Amplification

The 18S rDNA was amplified using PCR with the Red dye Taq 2x Mastermix, Amplicon. It was aimed to obtain a 666 bp fragment of the 3' half of the 18S rRNA genes. PCR conditions included an initial denaturation step at 94 °C for 5 min followed by 30 cycles for 30 s at 94 °C, 30 s for annealing at 55 °C and 60 s for extension at 72 °C. A final

extension step was carried out for 5 min at 72 °C. The primers details used for the amplification of the 666 bp fragment were: forward, 5' ATACATGAGCAAAAT CTCAAC-3'; reverse, 5'-CTTATTATTCCATGCTGCAG-3' (Inokuma, *et al.* 2002) [9]. PCR was done by based on the protocols followed by Szabo *et al.* (2005). All PCR reactions were carried out with a thermocycler (T100 thermocycler™, BioRad) at a final volume of 20 µl. Following PCR, 1% agarose-gel electrophoresis was used to verify for amplified fragments by comparing them to a molecular ladder (MX-0917-01 100bp DNA Ladder™, Medox).

Data submission in GenBank

The 18S rRNA sequence of tick samples were submitted in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) to record each sequence with accession number.

Results

Molecular identification of *H. canis* by 18S rRNA gene

The genomic DNA samples of *R. sanguineus* ticks were screened by PCR using specific primers of 18S rRNA in accordance with documented length of amplification fragments in tick species the expected length for the 18S rRNA fragment was approximately 666 bp (Figure 1).



Fig 1: Gel picture showing 18S rRNA amplicon of *H. canis* on 1.5% agarose gel stained with ethidium bromide, Lane M: 100bp DNA ladder, Lane 12 is positive with product size of approximately 666 bp

Discussion

To detect *H. canis* infection in *R. sanguineus* ticks, PCR amplification and sequencing was carried out to obtain a 666 bp fragment of the 18S rDNA sequences (Figure 1). The resulting nucleotide sequences were compared with similar sequences available in GenBank using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Sequence Tool (BLAST) search engine (<http://blast.ncbi.nlm.nih.gov/blast.cgi>). The 18S rRNA gene sequence of *H. canis* showed 98.10 percent homology with other isolates of *H. canis* available in the gene bank.

Out of the 324 ticks analysed, *H. canis* DNA was detected in pooled samples of *R. sanguineus* ticks collected from Chennai (15 per cent), Madurai (25 per cent) and Namakkal (20 per cent) respectively. Similar results were obtained by (Dantas-Torres 2008, Aktas *et al.* 2013, Giannelli *et al.* 2013 and Aktas and Ozubek, 2017) [4, 1, 7, 1]. This study confirmed the presence of *H. canis* infections in *R. sanguineus* ticks from three agro climatical zones of Tamil Nadu. Further research is

required to gain more insights into the global transmission of *H. canis* by *R. sanguineus* ticks.

Conclusion

This study confirmed the presence of *Hepatozoon canis* in *Rhipicephalus sanguineus* sensu lato ticks collected from three major districts of Tamil Nadu, with infection rates of 15%, 25%, and 20% in Chennai, Madurai, and Namakkal, respectively. Molecular detection using 18S rRNA PCR and sequencing revealed a 666 bp amplicon showing 98.10% homology with known *H. canis* isolates, validating the reliability of the assay. These findings highlight the significant role of *R. sanguineus* ticks as vectors in the regional transmission of canine hepatozoonosis. Understanding tick-borne pathogen distribution is essential for improving diagnostic, preventive, and control strategies. Future studies should explore seasonal dynamics, vector competence, and broader epidemiological patterns across India.

Conflict of Interest

Not available

Financial Support

Not available

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