Full Length Article

MOLECULAR DETECTION OF *ANCYLOSTOMA CANINUM*: A PREDOMINANT ZOONOTIC HOOKWORM THAT AILS DOGS IN CHENNAI, TAMIL NADU, INDIA

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ABSTRACT

Hookworms of dogs especially Ancylostoma caninum and A.ceylanicum are the predominant gastro intestinal parasites of zoonotic significance. These are soil transmitted helminths (STH) whose larvae dwell in the soil and are capable of penetrating the host skin and then establishing itself in the intestine causing gastro-enteritis and anaemia. Both A.caninum and A.ceylanicum can infect dogs and their zoonotic potential is different and speciation becomes a necessity especially since it is difficult to distinguish the species morphologically. The current study was envisaged for molecular speciation of the hookworms affecting dogs in Chennai. A total of 185 faecal samples were examined of which 58 (31.35%) were positive samples and these were subjected to molecular speciation. The primers targeting ITS-1 gene of the parasite were designed and it was found that A.caninum was the predominant species prevalent amongst dogs in Chennai.

Keywords: Hookworm infection, dogs, *Ancylostoma caninum,* PCR, India, Asia

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INTRODUCTION

Infection with gastrointestinal nematodes causes chronic morbidity in dogs. Hookworm infections are common in dogs which can result in severe anaemia due to their blood feeding habits. Of the hookworms infecting dogs, *Ancylostoma caninum* and *A.ceylanicum* are the most common species that have been reported from the Asian continent (Phosuk *et al.,* 2013). These parasites are of zoonotic significance as they can cause cutaneous larval migrans along with *A.braziliense* of cats. Though man serves as dead end host for *Ancylostoma caninum* where the larvae are limited to the prickle cell layer, reports suggest that *A.ceylanicum* can establish in man and result in jejunitis (Traub, 2013).

The species of zoonotic hookworm of dog in the Asian continent has been reported to be predominantly *A.ceylanicum* (Traub, 2013; Strake *et al.,* 2020). Molecular

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speciation in Sri Lanka, Myanmar, Malaysia and Thailand has identified the presence of *A.ceylanicum* in human populations (Aung *et al.,* 2017; Phosuk *et al.,* 2013). Traub (2013) indicated that the dog species in the Asian continent was *A.ceylanicum* while *Ancylostoma caninum* was not so rampant. Since speciation of *Ancyclostomes* of canines has not been undertaken in the Indian subcontinent the current study was envisaged for molecular speciation of the hookworm species occurring in dogs. As the ITS-regions of ribosomal gene cluster is highly conserved regions of the species belonging to this genus, primers targeting these regions were designed and the molecular speciation was carried out.

MATERIALS AND METHODS

Collection of faecal sample and DNA extraction

A total of 185 faecal samples from dogs presented to the Small Animal Veterinary Unit of the Clinical Complex of Madras Veterinary College were examined during a 9 month study period spanning from August, 2020 to April, 2021. Of the 185 samples, 58 faecal samples were found to be positive for the presence of typical hookworm eggs by flotation technique based on morphometry. These samples were then subjected to DNA extraction using QIAGEN stool mini kit® (Germany). Briefly, the faecal samples were concentrated by sedimentation technique and the sediment was utilized for DNA extraction. The faecal sediment was subjected to bead beating followed by a freeze - thaw process (freezing at -20°C and thawing at room temperature: approximately 37°C) in order to maximize the DNA yield with slight modifications of the protocol followed by Ayana *et al*. (2019). The DNA extraction was then carried out as per the manufacturer's protocol.

Extraction of larval and adult DNA

Representatives of faecal samples positive for hookworm eggs were cultured by Haradamori method (Nongmaithem *et al.,* 2019). The third stage larvae (L3) were collected on day 5 of culture and single larva was isolated. DNA extraction was carried out using QIAGEN Blood and tissue mini kit®(Germany).

Adult hookworms were collected at the Department of Veterinary Pathology, Madras Veterinary College during necropsy from a 6 month old Labrador pup (Fig.1) were identified based on standard morphological keys as *Ancylostoma caninum* (Traub *et al.,* 2021). The DNA was extracted from these worms and was used as positive control for the PCR assay.

Polymerase chain reaction

PCR was carried out individually in all samples which showed the presence of Ancylostoma eggs to identify the species of hookworms infecting dogs viz., both *Ancylostoma caninum* and *A.ceylanicum* using both published set of primers (Hu *et al.,* 2015) and primers designed using Primer-3 plus software targeting the ITS-1 gene of the species. The sequences of the primers designed in this study were as follows (Table 1):

The conditions for PCR were as per Hu *et al.,* (2015). For the designed set of primers the conditions were: Initial denaturation at 94°C, followed by denaturation at 94°C for 30 sec, annealing at 50°C for 40 sec, extension at 72°C for 25 cycles and final extension at 72°C for 5 min. In case of *A.ceylanicum*, the conditions were same except annealing was carried out at 56°C. The PCR products were then electrophoresed using 1.5% agarose gel. Sequence analysis of the PCR products was also done.

RESULTS AND DISCUSSION

The electrophoresis of PCR products showed that *Ancylostoma caninum* is the only species of hookworm infecting dogs in Chennai. In PCR assays with both published as well as designed primers, only *Ancylostoma caninum* was amplified with both the egg and larval DNA (Fig. 2 and Fig. 3). Nucleotide BLAST analysis of the PCR products revealed 100 per cent homology with *Ancylostoma caninum* strains available in GenBank. The sequences were submitted in GenBank and the accession numbers assigned were OM108318 and OM108319.

Hookworms in dogs especially those belonging to the genus *Ancylostoma* gain significance due to their zoonotic potential. In the genus *Ancylostoma, Ancylostoma caninum* and *A.ceylanicum* commonly infect dogs. These soil transmitted helminths, are termed as 'terror in the dirt'(Bryant and Hallem, 2018) due to their ability to infect human causing cutaneous larva migrans (CLM). Of the two species, *A.ceylanicum* is capable of establishing itself in the digestive system of humans causing hookworm disease (Traub *et al.,* 2013).

Numerous reports suggest that *A.ceylanicum* is the second most common hookworm species infecting humans in Southeast Asia and Oceania (Traub *et al.,* 2013). The reports also suggest that it is the most underestimated and neglected disease of humans and dogs play a major role in its transmission (Gerber *et al.,* 2021).

This study was undertaken for molecular speciation of the hookworms infecting dogs in Chennai. Previous studies have reported that hookworm infections were most common in well-maintained and nourished dogs which remain apparently normal despite of heavy infections which may be due to their ability to compensate the blood loss. However, such asymptomatic animals pose a greater risk to the human population.

The aim of this study was to bring out the implications of the possible zoonotic significance of these hookworm infections in dogs. Speciation thus becomes imperative, since *Ancylostoma caninum* does not reach patency in human beings and is considered to be of lesser zoonotic significance compared to *A.ceylanicum*.

The results of this study suggest that *A.caninum* is the predominant species prevalent in dogs in Chennai. The findings of Rehman *et al.,* (2017) stated that *Ancylostoma caninum* was the predominant species infecting dogs in Pakistan concurs with the findings of the current study. This implies that an existing gap of those

hookworm infections affecting humans and those found in dogs requires to be bridged in order to interpret the role of dogs in transmission of these parasites.

The findings of the current study suggest that more detailed insights and holistic approach is required to understand the prevalence of the hookworm species present in the tropics and its implications on animal and human health. Further studies are underway to identify other hookworm species like *Ancylostoma* braziliense affecting canines in Chennai.

Conflict of interest

The authors certify that there is no actual or potential conflict of interest in relation to this research.

Table 1: The primer sequence designed using Primer-3 plus

Target species	Name of the primer	Sequence (5'-3')	Product size
A.ceylanicum	A.cey.DF	CTACGTGAGGTGTCTATGTG	449 bp
	A.cey. DR	GTGTCACAAGCTACACTGTA	
A.caninum	A.can. DF	AGCCCTATGTAAGGTGTCTA	301 bp
	A.can. DR	CGAATCGACCGATCCATC	

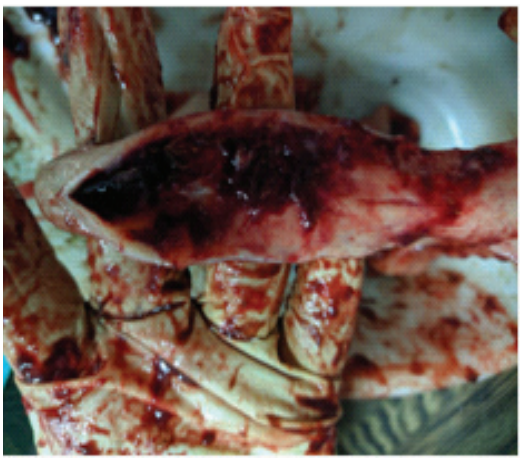

Fig. 1: *Ancylostoma* worms recovered from intestine of pup at necropsy

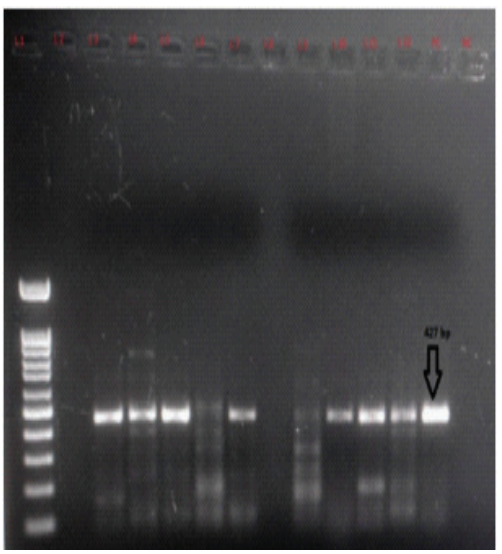

Fig. 2: Agarose gel electrophoresis of PCR products. Lane 1: Ladder, L2: blank, L3-L12: samples, L13: Positive control and L14:Negative control. Product size: 427 bp

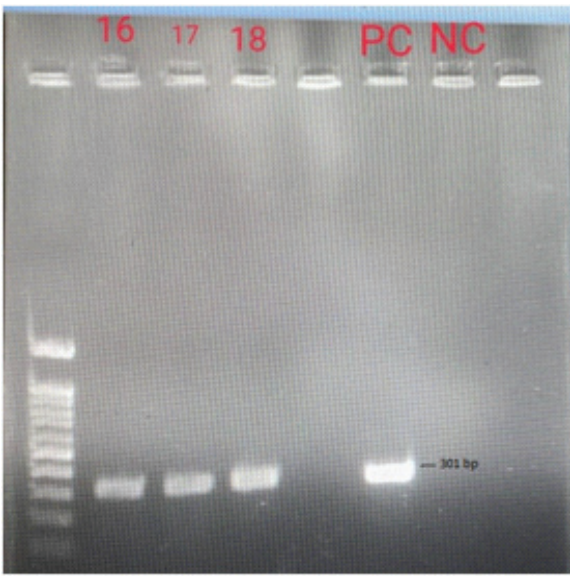

Fig. 3: Agarose gel electrophoresis of PCR products. Lane 1: Ladder, L2-L4: samples 16-17, L5: blank, L6: Positive control and L7: Negative control. Product size: 301 bp

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