RESEARCH ARTICLE

Prevalence of Canine Gastrointestinal Helminths and Molecular Confirmation of *Toxocara canis*

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Abstract

The present study was carried out to assess the prevalence of various canine gastro-intestinal (GI) helminths in animal population and soil in and around Tirupati city of Andhra Pradesh (India), and to confirm *Toxocara canis* at molecular level through Polymerase Chain Reaction. A total of 196 faecal and 111 soil samples were collected and examined. The overall prevalence of canine GI helminths among animal population and soil was 54.08% and 41.44%, respectively. The faecal samples were confirmed with 4 canine GI nematodes, *viz., Ancylostoma* spp. (20.41%), *Toxocara canis* (16.84%), *Strongyloides stercoralis* (5.61%), *Trichuris vulpis* (3.06%) and, one canine GI cestode, *viz., Dipylidium caninum* (3.57%) and combinations of *Ancylostoma* spp., *Toxocara canis*, *Strongyloides stercoralis* (4.59%), while the soil samples had *Toxocara canis* (19.81%), *Ancylostoma* spp. (14.14%), *Strongyloides stercoralis* (5.40%) and *Trichuris vulpis* (1.80%). Location wise, the prevalence of soil transmitted canine GI helminths was the highest in public parks (46.60%) followed by veterinary dispensaries/ hospitals (44.40%), school grounds (40.00%) and temple surroundings (12.50%). The amplification of genomic DNA (extracted from adult worms and eggs of *Toxocara canis*) through PCR targeting the Internal Transcribed Spacer-2 region of ribosomal DNA and subsequent gel documentation exhibited a ~540 bp product confirming the *Toxocara canis.*

Key words: Canine gastrointestinal helminths, Polymerase chain reaction, Prevalence, *Toxocara canis.*

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Introduction

The dog (*Canis familiaris*) is the most common pet animal in India, with 63% of pet owners owning a dog (Fowler, 2024). The contaminated soil with canine helminths or their eggs/larvae is the most important route of transmission of zoonotic helminths to humans, and presence of parasitic forms (eggs and larvae) in the soil is the most direct indicator for the local population's risk.

The *Toxocara* is a genus belongs to the super family Ascaridoidea. It is oviparous and exhibits direct life cycle with development of eggs occurring in the environment without hatching and the definitive host getting infection by ingestion of embryonated eggs. Adult dogs act as reservoir host for *Toxocara canis* and source of infection to newborn puppies which in turn play an important role in spreading the infection to humans by contamination of surrounding environment with parasitic eggs (Cruz *et al.,* 2008). The human toxocariasis which is manifested in the form of visceral larva migrans (VLM), is primarily caused by *Toxocara canis*. This particular organism does also exhibit vertical mode of transmission. Since the first human case of VLM recorded in 1992, a significant number of VLM cases have been reported throughout India (Thakkar *et al*., 2012; Kaur *et al*., 2017). Therefore, there is a specific need to study the organism with respect to its prevalence and soil contamination level. The present study was aimed at assessing the prevalence of various canine gastrointestinal helminths in animals and environment with a special focus on the *Toxocara canis* in and around Tirupati city of Andhra Pradesh using molecular techniques such as PCR.

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Materials and Methods

The present investigation was carried out at the Departments of Veterinary Public Health and Epidemiology, and Veterinary Parasitology, College of Veterinary Science, Tirupati (India) to assess the prevalence of various canine gastrointestinal helminths in and around the Tirupati city of Andhra Pradesh

which had a climate with a distinct dry season followed by a wet season. Summer temperatures range from 26°C to 45°C, while winter temperatures range from 12°C to 31°C.

A total of 196 freshly voided faecal samples of 3-5 grams each were randomly collected using a spatula from dogs that were brought to veterinary hospitals, animal birth control centres and rescue homes in and around Tirupati, and transferred to sterile plastic containers in case of nearby places. The samples were preserved in 95% ethanol in case of faraway places as suggested by Papaiakovou *et al.* (2018). They were labelled immediately with details of dog type (stray/pet), place, date of collection and deworming status of the dog. Cold chain (4°C) was maintained during transporting of the samples to lab. The samples intended for molecular analysis were frozen at -20°C till use (Song *et al.,* 2016). The faecal samples were thoroughly examined grossly for the presence of adult parasites. Then they were screened using the direct smear, sedimentation and floatation techniques to detect the eggs of gastrointestinal parasites.

A total of 111 soil samples of 200 grams each were randomly collected at a depth of 3-5 cm using a shovel from specific locations such as public parks, play areas, animal habitation areas, garden soils, veterinary hospitals and school grounds, and deposited into polythene bags. All the soil samples collected were processed by Lorcain (1994) method and then the eggs/larvae were collected through sedimentation of floating fluid.

The faecal and soil samples positive for *Toxocara canis* were subjected to molecular confirmation, for which the genomic DNA was extracted using QIAGEN QIAamp® DNA FFPE power kit (bought from Qiagen) both from its adult worms which were dioecious, with males 4-6 cm longer than females, and its eggs which were brownish in colour and nearly spherical. Eluted gDNA was tested for its concentration and purity with Nanodrop analysis. The purity of the gDNA sample was estimated by observing the ratio between optical density (OD) value at 260 and 280 nm. The DNA samples were considered to be of sufficient purity if the ratio was 1.7 and above. The eluted gDNA samples with sufficient purity were stored at -20°C for further use. Molecular confirmation of *Toxocara canis* was carried out with oligonucleotide primers (Forward primer NC13- 5′ ATCGATGAAGAACGCAGC 3′ and reverse primer NC2- 5′ TTAGTTTCTTTTCCTCCGCT 3′) by targeting the Internal Transcribed Spacer-2 (ITS-2) region of ribosomal DNA (rDNA) by employing PCR (Khademvatan *et al.*, 2013). The primers were custom synthesized from Eurofins Genomics India Pvt. Ltd., Bangalore. Each PCR tube was filled with a reaction mixture of 25 µL composed of forward primer (1.0 µL), reverse primer (1.0 µL), Emerald Amp® GT PCR master mix (2X) (12.5 µL), nuclease free water (8.5 µL) and sample DNA (2.0 µL). The protocol of PCR involved subjecting the PCR mixture to initial denaturation at 94°C for 3 min, application of a cycle of denaturation at 94°C at 30 sec, annealing at 53.5°C for 35 sec and elongation at 72°C for 1 min 35 times, leaving the product at 72°C for 10 min for final elongation, and holding the end product at 4°C until its removal from the thermocycler. The final PCR products were preserved at -20°C for further analysis. Both negative (mix inoculated with ultra-pure water in substitution of DNA in equal volume) and positive (mix inoculated with genomic DNA extracted from adult worms) controls were run along with the test samples.

The amplified PCR product was subjected to electrophoresis as described by Sambrook and Russel (2001) in submerged gel electrophoresis apparatus (obtained from Genei, Bengaluru). The bands were visualized at 300 nm wavelength using a gel documentation system (BIO-RAD, USA) and the photographs were obtained using its image lab software. Size of the amplified PCR product was determined by comparing with a standard molecular weight marker (100 bp DNA ladder).

Results and Discussion

Prevalence of Canine Gastrointestinal Helminths among Faecal Samples

The study revealed that the overall prevalence of canine gastrointestinal helminths in and around the Tirupati city of Andhra Pradesh was 54.08%. It was higher in stray dogs (38.77%) than the pet dogs (15.30%), and lower in dewormed dogs (9.09%) than the ones that were not dewormed (90.74%). The overall prevalence obtained in the present study was similar to that of Roja *et al.* (2019). On the other hand, it was lower than the results of Ahmad *et al.* (2018). Further, Vatsya *et al.* (2010), Singh *et al.* (2012) and Qadir *et al.* (2012) reported lower prevalence rates of canine GI helminths than the current study. The faecal samples were confirmed with 4 canine GI nematodes, *viz., Ancylostoma* spp. (20.41%), *Toxocara canis* (16.84%) (Fig. 1, 2), *Strongyloides stercoralis* (5.61%) (Fig. 3), *Trichuris vulpis* (3.06%) (Fig. 4) and, one canine GI cestode, *viz.*, *Dipylidium caninum* (3.57%) (Fig. 5) and combinations of *Ancylostoma* spp., *Toxocara canis*, *Strongyloides stercoralis* (4.59%) (Fig. 6) which has zoonotic importance. Among the total mixed infection samples, combination of *Ancylostoma* spp. and *Toxocara canis* (77.78%) was more prevalent than that of *Toxocara canis* and *Strongyloides stercoralis* (22.22%). These findings were in accordance with those of Sudan *et al.* (2013) and Roja *et al.* (2019), who also identified the *Ancylostoma* spp. followed by *Toxocara canis* as the major parasites in faecal samples. However, Moudgil *et al.* (2016) and Sharma *et al.* (2006) reported *Toxocara canis* as the most prevalent parasite in dogs. The finding that prevalence of single parasitic infections (49.49%) being higher than that of mixed infections (4.59%) was consistent with reports of Katagiri and Oliveira (2008), Mukaratirwa and Singh (2010), Qadir *et al.* (2012), and Sudan *et al.* (2013). The prevalence rates of *Strongyloides stercoralis* (5.61%) and *Trichuris vulpis* (3.06%) were almost similar to those reported by Moura *et al.* (2010).

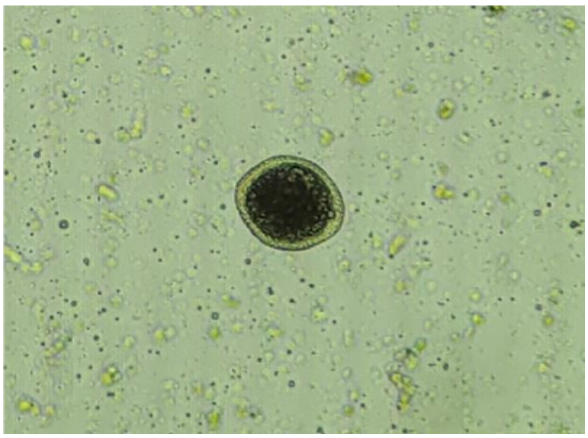

Fig. 1: Egg of *Toxocara canis* from a faecal sample (10X)

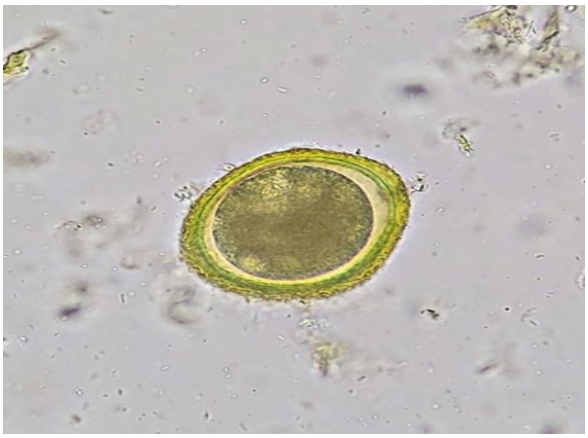

Fig. 2: Egg of *Toxocara canis* from a faecal sample (40X)

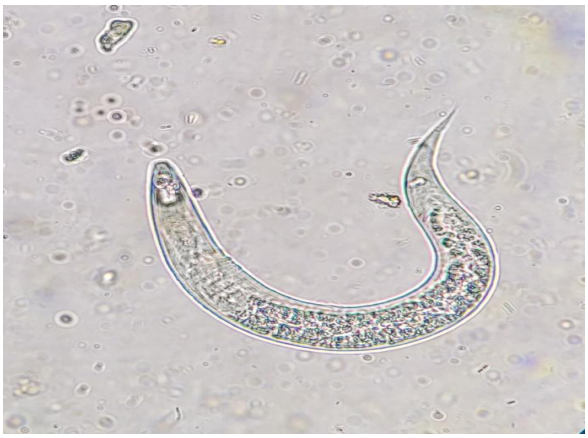

Fig. 3: Larva of *Strongyloides stercolaris* from a faecal sample (40X)

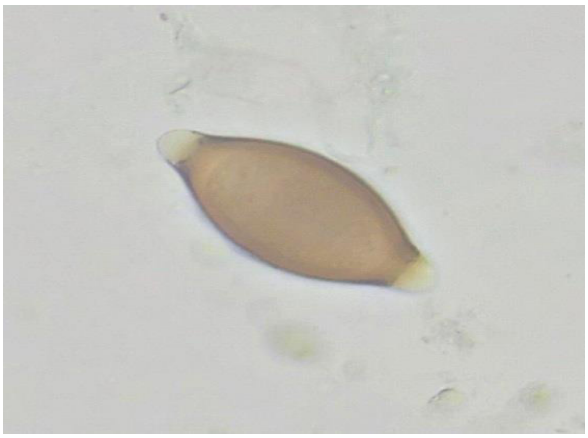

Fig. 4: Egg of *Trichuris* spp. from a faecal sample (40X)

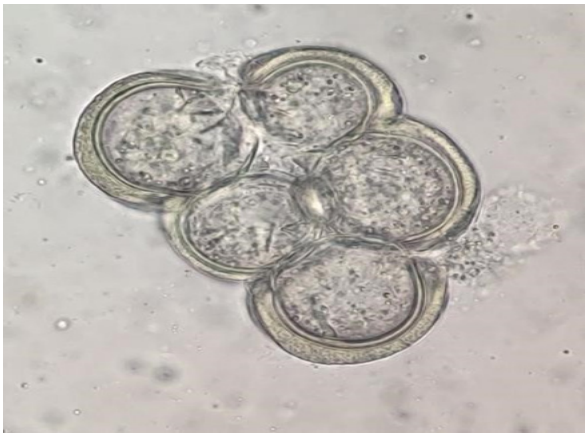

Fig. 5: Eggs of *Dipylidium caninum* eggs from a faecal sample (40X)

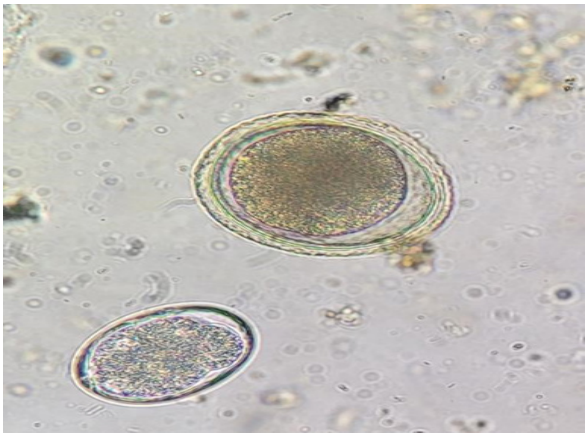

Fig. 6: Eggs of *Toxocara canis* and *Strongyloides stercolaris* from a faecal sample (40X) (Mixed infection)

Prevalence of Canine GI Helminths among Soil Samples

The magnitude of soil contamination in a region greatly depends on ecological conditions, aggregated distribution of infected host populations and living standards of the people (Qadri, 2008). It was found in the present study that the overall prevalence of soil transmitted canine gastrointestinal helminths in and around the public places of Tirupati was 41.44%. A total of 4 canine GI nematodes, *viz.*, *Toxocara canis* (19.81%), *Ancylostoma* spp. (14.14%), *Strongyloides stercoralis* (5.40%) and *Trichuris vulpis* (1.80%) were found in the soil samples collected in the present study. Moura *et al.* (2010) reported similar findings. Location wise, the prevalence of soil transmitted canine GI helminths was the highest in public parks (46.60%) followed by veterinary dispensaries/ hospitals (44.40%), school grounds (40.00%) and temple surroundings (12.50%).

Molecular Confirmation of *Toxocara canis*

The yield of gDNA from eggs of *Toxocara canis* from faecal samples ranged between 124.3 ng/µL and 196.7 ng/µL, while that from soil samples ranged between 76.4 ng/µL and 167.2 ng/µL. The quantity of gDNA extracted from the adult worms was 765.6 ng/µL. Chen *et al.* (2012) stated that PCR can be used for confirmation of *Toxocara canis*. The present study revealed that the gDNA extracted from eggs in faecal and soil samples, upon amplification targeting the ITS-2 gene of DNA of *Toxocara canis,* yielded a ~540 bp product (Fig. 7), which was a complete ITS-2 sequence guarded by 5.8S and 28S ribosomal DNA (rDNA) conforming *Toxocara canis*. The absence of size variation on agarose gels indicated towards confirmation of all the amplicons as *Toxocara canis*. These findings coincided with those of Charitha (2018).

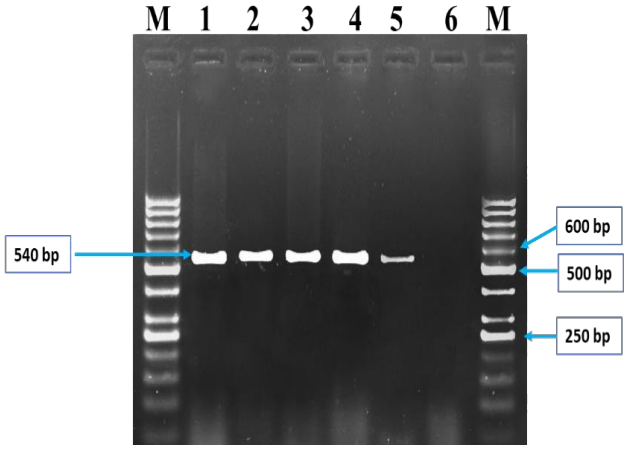

Fig. 7: PCR amplification product of ITS-2 rDNA from faecal and soil samples positive for Toxocara canis (Lane M - Molecular weight marker (100 bp), Lane 1-Positive control of Toxocara canis from adult worm DNA, Lane 2 & 3-Toxocara canis positive sample from dog faecal sample, Lane 4 & 5-Toxocara canis positive sample from soil sample, and Lane 6-Negative control)

Conclusion

Based on the results of the present study, it can be concluded that *Toxocara canis* and *Ancylostoma* spp. are the major canine gastrointestinal helminths with higher prevalence rates in animal population and soil in around the study area, and that the former can be confirmed at molecular level by observing ~540 bp product in gel documentation system after amplification of gDNA from the samples.

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