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Original Article

Toxocara Nematodes in Stray Cats from Shiraz, Southern Iran: Intensity of Infection and Molecular Identification of the Isolates

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Abstract

Background: *Toxocara* is a common nematode of cats in different parts of Iran. Despite the close association of cats with human, no attempt has been done so far for molecular identification of this nematode in the country. Therefore, current study was performed on identification of some isolates of *Toxocara* from stray cats in Shiraz, Fars Province, Southern Iran, based on morphological and molecular approaches, and also determination of intensity of infection.

Methods: This cross-sectional study was carried out on 30 stray cats trapped from different geographical areas of Shiraz in 2011. Adult male and female worms were recovered from digestive tract after dissection of cats. Morphological features using existing keys and PCR-sequencing of ITS-rDNA region and *pcox1* mitochondrial 1 gene were applied for the delineating the species of the parasites.

Results: Eight out of 30 cats (26.7%) were found infected with *Toxocara* nematodes. All the isolates were confirmed as *Toxocara cati* based on morphological features and the sequence of ribosomal and mitochondrial targets. Intensity of infection ranged from one to a maximum of 39 worms per cat, with a mean of 10.25 ± 12.36 , and higher abundance of female nematodes.

Conclusion: The most prevalent ascaridoid nematode of stray cats in the study area was *T. cati* and female nematodes were more abundant than that of males. This issue has important role in spreading of eggs in the environment and impact on human toxocarasis.

Introduction

Toxocara species are common ascaridoid nematodes of cats and dogs throughout the world. They are causative agents of toxocariasis, a zoonotic parasitic disease in human with worldwide distribution. The most widespread species of *Toxocara* in dogs and cats are *Toxocara canis* and *Toxocara cati*, respectively (1). Humans are infected by the ingestion of *Toxocara* eggs from contaminated soil, unwashed hands or raw vegetables. The larvae emerge in the intestine and migrate to muscle and neurological tissues, where they can remain for many years without growth, differentiation or reproduction (2). Some peoples may be infected by eating the larvae present in undercooked meat of infected paratenic hosts such as chickens, sheep and cattle or earthworm (3-6). The clinical symptoms of toxocariasis depend on where in the body infected. There are several forms of toxocariasis, namely visceral larva migrans, ocular larva migrans, covert toxocariasis, and neurotoxocariasis (7-10).

Recent epidemiological studies demonstrated the widespread prevalence of human infection with *Toxocara* in the world (7, 11-16). *Toxocara* in cats has important role on human health. Prevalence of *T. cati* in cats has been estimated to vary from 0.8 to 59.3% in different parts of the world (17-26). In Iran, cats live freely in urban and rural areas, discharging *Toxocara* eggs in the environment which are transmittable to human. There are some reports of contamination of the soil in public areas with *Toxocara* eggs in Iran (27-30). The prevalence of *T. cati* in cats ranges from 8% to 52.8% in different parts of Iran (31-38). Shiraz, Fars Province, Southern Iran is a city with high prevalence of *Toxocara* infection in cats (31, 32), as well as seroepidemiological evidence of high prevalence of toxocariasis in school children (39). Nevertheless, no attempt has been done so far for molecular identification of this nematode from cats in this city or any other endemic areas in the country.

Therefore, current study was performed on identification of some isolates of *Toxocara* from stray cats in Shiraz, based on morphological and molecular approaches, and also determination of intensity of infection.

Materials and Methods

Sample collection

This cross-sectional study was carried out in Shiraz, capital city of Fars Province, situated in southern Iran. Cats were captured from different geographic areas of the city, from February to November 2011. Pregnant females were excluded from this study. Overall 30 cats were anaesthetized by intra muscular injection of high doses of anesthetic drug (Ketamine, 15-40 mg/kg) and then were humanely euthanized by chloroform. Their digestive systems were removed and the intestinal contents were examined for the presence of *Toxocara* nematodes. Recovered helminthes were washed extensively in physiological saline and kept in ethanol alcohol 70% for further examinations. This research project was reviewed and approved by the Ethics Committee of Tehran University of Medical Sciences, Iran.

Morphological studies

The mature male and female worms were identified according to the morphological features using existing keys and descriptions (40, 41). The female worms were distinguished by their eggs, that are brown and pitted, and males by their posterior end which is curved with paired spicules, showing a prominent point at the tail-end.

Molecular studies

Total genomic DNA was extracted using Qiamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The mitochondrial and ribosomal DNA regions were subjected to PCR amplification

by forward (JB3:5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and reverse (JB4.5:5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') primers for amplification of mitochondrial gene (*pcox1*) (42); and forward (NC5:5'- GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and reverse (NC2:5'- TTA GTT TCT TTT CCT CCG CT-3') primers for amplification of internal transcribed spacer (ITS) region in ribosomal DNA (43). The PCR products were electrophoresed on agarose gel and the DNA bands were visualized using a UV transilluminator and photographed. All PCR products of *pcox1* gene and four PCR products of ITS fragment were purified with PCR purification Kit (BIONEER, Korea) according to manufacturer's instructions. Purified products were submitted to sequencing in two directions using the same forward and reverse primers employed in the PCR. Nucleotide sequences were compared

with GenBank sequences using the BLAST system (<http://www.ncbi.nlm.nih.gov/>).

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (Statistical Package for Social Science). All statistical tests were expressed as significant at 95% confidence interval.

Results

The collected *Toxocara* worms were identified in the laboratory based on morphological features. All samples had very broad cephalic alae (Fig.1, A). The male nematodes had a curved posterior end with paired spicules, showing a prominent point at the tail-end which was distinguishable from the straight-tailed female nematodes (Fig. 1, B and C). In the female worms, the egg shell of *Toxocara* was pitted (Fig.1, D).

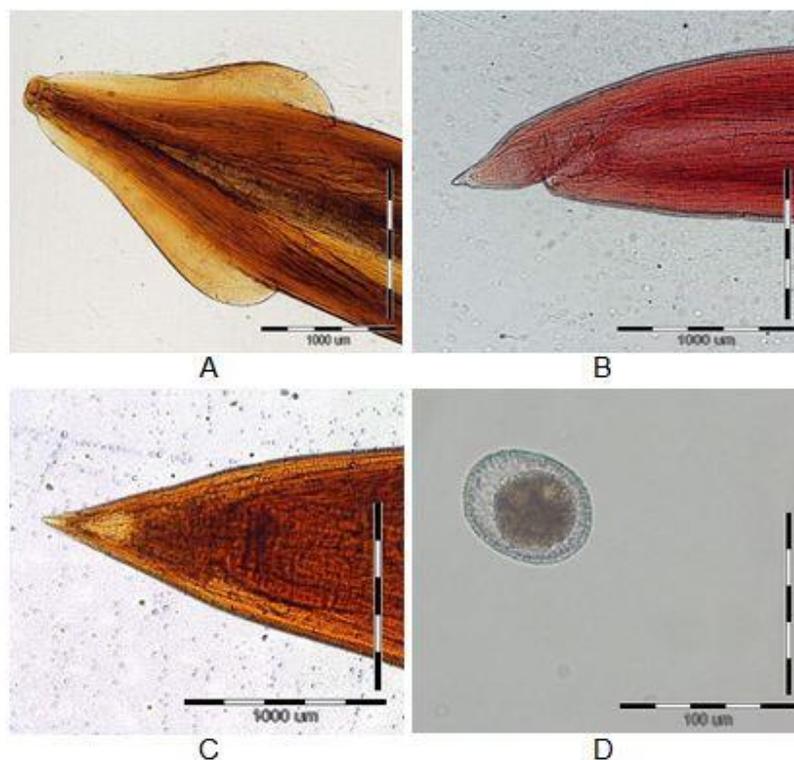


Fig. 1: The cephalic alae of *Toxocara cati* (A), the tail-end of *Toxocara cati* male (B), the tail-end of *Toxocara cati* female (C), Egg of *Toxocara cati* (D)

A, B, C: Bars indicate 1000 µm. / D: Bars indicate 100 µm.

Morphologically, the genus of all these *Toxocara* isolates was identified as *T. cati*. Then, the isolates were characterized by amplification of the *pcox1* gene and ITS fragment. For all of them, amplicons of about 450 bp for the *pcox1* gene and 1100 bp for ITS fragment were successfully produced by PCR (Fig. 2). The sequences were achieved and com-

pared with other available sequences in GenBank. By using the BLAST system either, all above mentioned isolates were identified as *T. cati*. The ITS sequences of four isolates obtained have been deposited in GenBank database (Accession numbers: JX53-6258-JX53-6261).

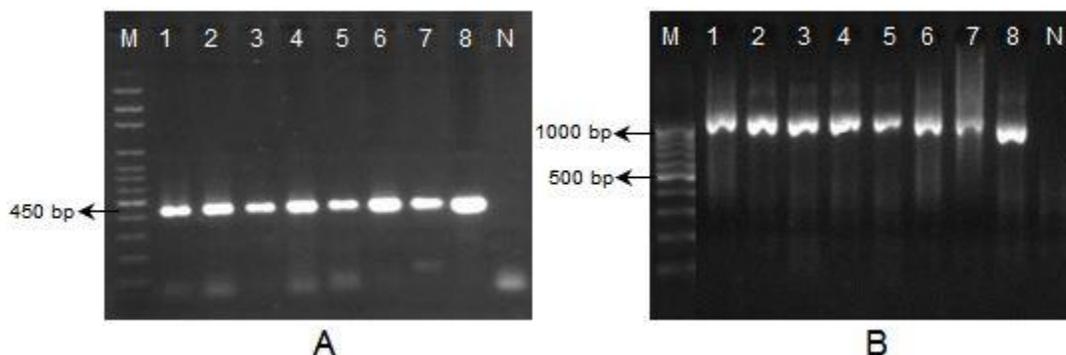


Fig. 2: Agarose gel electrophoresis of *pcox1*-PCR (A) and ITS-PCR (B) products from *Toxocara* isolates, M: 100 bp DNA Marker, N: negative control

Overall, 30 cats were examined in this study. As the relationships of age or sex of the animals and their infectivity with *Toxocara* was not the aim of this study, pregnant females were not enrolled in the study; therefore, 22 cats were male and the rest females. In general, 8 out of 30 stray cats (26.7%) were found in-

fectured with *T. cati* (Table1). The intensity of infection ranged from one to a maximum of 39 worms per cat, with a mean of 10.25 ± 12.36 (Table2). The mean intensity of infection with female and male nematodes was 7.125 and 3.125, respectively. No infectivity with *Toxascaris leonina* was found in these cats at all.

Table 1: Infection rate of *Toxocara cati* in stray cats in Shiraz, Iran

	Number	Percent	95% Confidence Interval	
			Lower	Upper
Infected cats	8	26.7	12.3	45.9
Non-infected cats	22	73.3	54.1	87.8
Total	30	100		

Table 2: Intensity of *Toxocara cati* infection in stray cats in Shiraz, Iran

	Statistic	95% Confidence Interval	
		Lower	Upper
Numbers of infected cats	8		
Numbers of <i>Toxocara</i> in each cat	Minimum	1	
	Maximum	39	
	Mean	10.25	4
	Std. Deviation	12.36	19.87

Discussion

In association with the infection rate of *T. cati* in stray cats collected from different parts of Shiraz City, this study showed that 8 out of 30 cats (26.7%) were infected. Sadjjadi et al. reported the prevalence of *T. cati* on 108 stray cats in Shiraz 52.8% (31). Another study showed that the infection rate of *T. cati* on 114 stray cats in Shiraz was 42.6% (32). In other studies from North (33, 34), Northwest (36) and Northeast of Iran (37), the prevalence of infection with *T. cati* was 8-44%, 8% and 28.8%, respectively. Similar studies have been done on prevalence of *T. cati* in central parts of Iran; for example in Kashan, 113 stray cats showed a prevalence of 13.3% (35). The prevalence of *T. cati* in Tehran was 9.4% (38). Although, investigation on the relationship between the prevalence of *Toxocara* and age or sex of the cats was not the aim of this study, however, previous studies showed that there was no significant difference in the prevalence of infection between male and female cats (31, 33, 34, 36, 37); and cats with less than 6 months old being more likely to be infected with *T. cati* than older cats (33, 37). Sadjjadi et al. reported the prevalence of infection was higher in younger cats compared to older animals; however, the difference was not significant (31).

In this study, the intensity of infection ranged from one to a maximum of 39 worms per cat, with a mean of 10.25. In a report from Shiraz, the mean intensity of infection with *T. cati* was 6.52 with a range of 1 to 50 worms per cat (31). Sharif et al. indicated that the intensity of infection ranged from 1 to 32 worms per cat, with a mean of 7.3 (33). In other study in stray cats from north of Iran, the mean intensity of infection with *T. cati* in cats was an average of 3 *T. cati* in each cat (34). Considering the sex of the *T. cati* recovered from the cats in the current study, female nematodes were more abundant than male ones with a mean of 7.125 and 3.125 nema-

toes per cat, respectively. This issue is important respect to the distribution of *T. cati* eggs in environment, because every female *Toxocara* shed about 200000 eggs per day (44) that will become capable of transmission to human and paratenic hosts after development in the soil.

Since the morphological identification of some ascaridoid species or their larval and egg stages is difficult; therefore some molecular methods using ribosomal and mitochondrial markers have been developed and used for accurate identification and diagnosis of such nematodes. As with the ascaridoid nematode of cats from Kuala Lumpur, Malaysia which based on morphology identified as *T. canis* (45), and characterized by the ribosomal DNA (rDNA) sequences as *Toxocara* sp. cf *canis* (46). Detailed morphological study of this nematode was done by Gibbons et al. and the parasite was described and named *Toxocara malaysiensis* (47). The first report of *T. malaysiensis* in cats outside Malaysia reported from China. The nematodes collected from cats in China were morphologically and genetically consistent with *T. malaysiensis* (48). These studies showed that this new species has a broader geographical distribution. According to these reports, molecular methods using ribosomal and mitochondrial sequences as genetic markers have been shown to provide reliable alternatives to more traditional methods for the specific identification of nematodes. In this study all ascaridoid nematodes isolated from cats based on morphological features identified as *T. cati*; and for confirmation using molecular approach, PCR amplification of the mitochondrial and ribosomal DNA and sequence analysis of the amplicons were applied on the same samples. Accordingly, all of the isolates were characterized by amplification of the mitochondrial (*cox1*) and ribosomal (ITS) genes as *T. cati*.

The result of this study is coincident with the previous studies in Shiraz (31, 32) indicating that the infection of cats with *Toxocara* nematodes in this city is considerable. The

high infection rate of *T. cati*, high intensity of infection and twice more abundance of female *T. cati* in cats in the present study emphasize that stray cats have important role in distribution of *Toxocara* eggs into the environment and their transmission to humans. Current diagnostic techniques cannot discriminate which species of *Toxocara* is responsible as causative agent of toxocariasis in human; therefore identification of parasite species in animals using molecular methods can be foundation for planning of prevention and control programs in human and animal communities.

Conclusion

The result of this study implies that *T. cati*, as the most prevalent acaridoid nematode of cats in the study area, might have the most important role in human toxocariasis in that area, but further studies on human cases will better clarify this issue. Similar studies on isolates from canid hosts and also from other geographical areas of Iran in recommended.

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