Original Article

Environmental Contamination of Different Areas of Isfahan Province of Iran with *Toxocara spp.* Eggs using Molecular Methods

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Abstract

Background: Toxocariasis is a parasitic disease caused by the larval stage of *Toxocara canis* and *Toxocara cati*. Infective stage of this parasite for human develops on soil. So, in this work contamination of the soil of public environments in five geographical areas of Isfahan province of Iran has been investigated. **Materials and Methods:** In this descriptive study, 355 soil samples were collected from parks, children's playgrounds, student dormitories, and university environments, and examined by Flotation method. The samples were then inspected using microscopic and molecular methods. **Results:** From the 355 examined soil samples in 77 (21.69%), and 87 (24.50%) cases *Toxocara* eggs were detected by microscopic and molecular methods, respectively. In the molecular method, 31 (8.70%) cases of *T. cati* and 44 (12.39%) cases of *T. canis* were identified. **Conclusion:** *Toxocara* eggs were identified in all areas of Isfahan province, although contamination rate was higher in Fereydun Shahr and Semirum counties.

Keywords: *Environmental pollution, Iran, Isfahan, polymerase chain reaction, Toxocara*

Introduction

Toxocara canis and *Toxocara cati* are nematode helminths of dogs and cats, respectively.[1]

Ova are excreted on the soil along with the animal's feces. After development on soil, if the eggs are eaten by humans, their larvae release in the intestine, migrate to different tissues, especially liver and cause human toxocariasis.[2] Several epidemiological studies have shown that soils of different areas such as parks, tourist regions, children's play stations, crowded and busy areas, slaughterhouses, and student dormitories are contaminated with (dogs and cats' *Toxocara* eggs). These contaminated soils can remain as sources of human infection.[3-10] *Toxocara* eggs are very resistant and usually survive in the cold winters for 6–12 months.[11] However, development and survival of the eggs are dependent on different environmental factors such as soil type, pH, light, temperature, humidity, and vegetation. The eggs on the soil are physically dispersed by rainfall, birds, beetles, earthworms, snails, and flies.[12-15]

Poverty, ignorance, eating soil, poor sanitation, presence of stray dogs and cats, and suitable climatic conditions are the factors in favor of transmission of *Toxocara* infection to humans.[16,17] Children are at a higher risk for toxocariasis due to more contact with the soil and the habit of eating and transfer of objects to their mouths.[18] Mice and birds feed on soil, so, they are easily infected as intermediate hosts. Final hosts become infected by eating these intermediate hosts.[19] Therefore, in the control program of *Toxocara* infections study of soil contamination is extremely important.[20] Numerous studies have been performed so far about soil contamination with *Toxocara* eggs in different parts of the world[21] and in Iran.[22] In the world, prevalence of *T. canis* in dogs and *T. cati* in cats were 1.2% and 3.2% in Australia, 4.4% and 4.6% in the Netherlands, and 6.1% and 4.7% in Germany respectively.[23] In Iran according to Maleki *et al.*[24] study, the average rate of soil contamination with *Toxocara spp* eggs was 16%, with the highest and lowest level of contamination in Tehran (38.7%), and Qazvin (3.15%) provinces, respectively. However, the situation of soil contamination in Isfahan province of Iran is not clear. Isfahan province with population of over five million located in the center of Iran

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and serves as a pass way from North to South and East to West of the country. Annually many domestic, and foreign tourists visit this province. Previous studies about soil contamination with *Toxocara spp*. in Isfahan were limited to certain areas. So, in this work soil of different counties and different locations such as parks, playgrounds, slaughterhouses, squares, and streets together have been examined.

Molecular methods were applied for better differentiation of *T. canis* and *T. cati* eggs.

Materials and Methods

In this descriptive research, study population consisted of soil samples that were collected from different areas of Isfahan province. The sample size for the whole province was 355, in which 115 were allocated to Isfahan (115 samples), and the remaining to 4 other selected counties including Ardestan, Semirum, Naein, and Fereydun Shahr (each county 60 samples).

Samples collection

Samples were collected from residential areas, tourist regions, busy places, parks, universities, student dormitories, slaughterhouses, and children's playgrounds. Isfahan is a large province, so considering climate, and geographical locations, five counties including; (Ardestan in the north), (Semirum in the south), (Naein in the east), (Fereydun Shahr in the west) and (Isfahan in the center) were selected.

Microscopic examination

In this study, for each sample, at least 250 gms of soil were taken from a depth of 5 cm and transferred to a plastic bag, and then transferred to the parasitology laboratory at Isfahan University of Medical Sciences. The soil samples were then filtered to remove the coarse particles. In the next step, the eggs of *Toxocara* parasite were isolated by flotation method (Sheather) and wet mount of each sample was observed under the microscope.

Molecular examination

DNA extraction

The parasite's eggs, which were collected, using saturated sugar solution, were broken using freeze-thaw and homogenizing methods (Bertin Instrument, Precelleys). Thereafter, DNA extraction was performed using the phenol–chloroform method.[24-26]

Polymerase chain reaction

For *T. canis*, forward primer (NC5: 5′-ATTAACGCGCAAG GTTGTGG-3′) and reverse primer (NC2: 5′-TGGCCATGCATTCCTCATTC-3′) and for *T. cati* forward primer (NC5: 5′-CTTCTGGTGCATTCTTT CGC-3′) and reverse primer (NC2: 5′- CCAAGCAACAA CAAACTACGC-3′) were designed by NCBI database and Genius Prime (Version 2019.2.1) software. The polymerase chain reaction (PCR) reactions were carried out in a 25 μL final volume, comprised 12.5 μL of PCR master mix (Amplicon, Denmark), 1 μL of each primer, 5 μL of template DNA, and 6.5 μL distilled water. Denaturation at 95 for 15 s, annealing at 61 for 30 s, and activation at 72 for 30 s were all used in the PCR procedures (Bio-RAD T100 thermal cycler, USA). After that, the PCR products were run on 1.5% agarose gel and visualized with UV detect equipment.

Results

In this study, 355 soil samples were examined by microscopic and molecular methods for detection of *Toxocara* eggs. In microscopic method, 77 samples were positive, details of these results have been reported in Table 1. Following molecular analysis (PCR), 87 samples were positive for *Toxocara spp*, 12 soil samples had mixed contamination Table 2. Semirum and Fereydun Shahr counties had the highest rate of contamination. Shape of *Toxocara* eggs in soil samples collected from Isfahan province following microscopic examination have been shown in Figure 1. Using the Chi-Square test, it was shown that *Toxocara spp*. The contamination rate was different in different counties and the difference was statistically significant (*P* value = 0.002).

Table 1: Prevalence of *Toxocara spp* eggs in soil samples in Isfahan province of Iran based on microscopic methods

Location	Total sample (No)	Positive (No)	Percent (%)
Ardestan	60	8	13.33
Semirum	60	17	28.33
Fereydun Shahr	60	21	35
Naein	60	7	11.66
Esfahan	115	24	20.86
Total	355	77	21.69

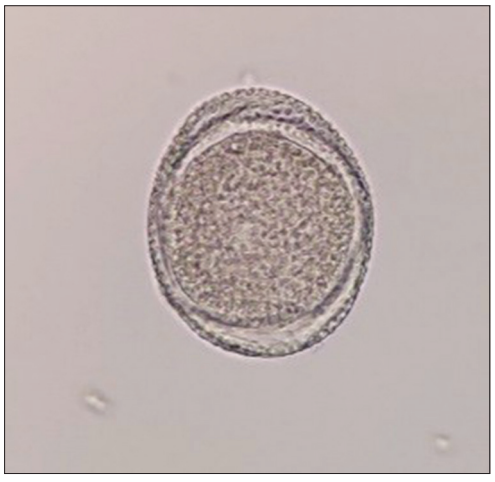

Figure 1: *Toxocara* parasite egg observed in soil samples collected from Isfahan province of Iran

The most contaminated areas, were parks and children's playgrounds for *T. cati* eggs and slaughterhouses for *T. canis* eggs [Table 3]. Using the Chi-Square test, there was no significant relationship between *Toxocara* eggs in soil and the sample location (*P* value = 0.083). Soil contamination with *Toxocara* eggs based on climatic conditions in different counties has been shown in Table 4. Using the Chi-Square test, it was shown that there is a difference in soil contamination rate among different climate conditions and this difference was statistically significant (*P* value = 0.001). Frequency map of soil contamination with *Toxocara spp* eggs has been shown in Figure 2. Results of PCR experiment following gel electrophoresis indicating 260bp band for *T. canis* and 204bp for *T. cati* have been shown in Figure 3.

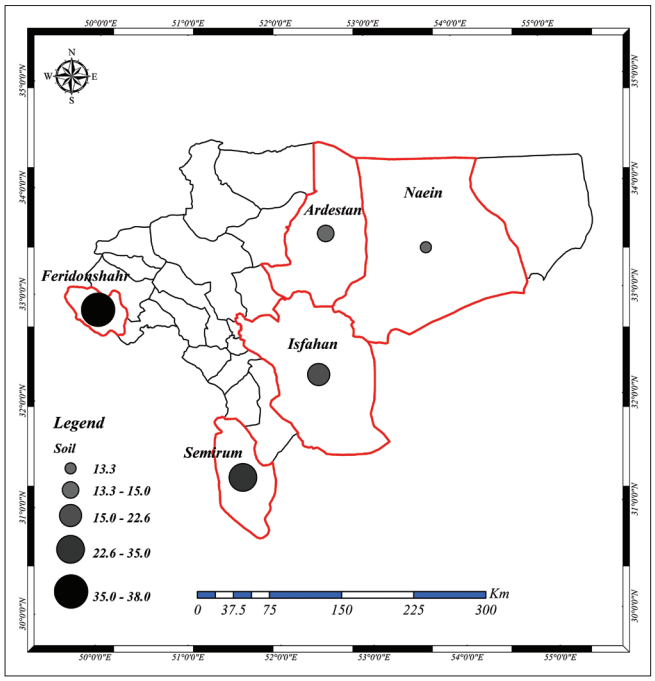

Figure 2: Frequency map of soil contamination with *Toxocara* eggs in different counties of Isfahan province

Discussion

Human toxocariasis is caused by the ingestion of infective *Toxocara* eggs in soil or soil materials. Different studies have also shown that the prevalence of toxocariasis in humans directly depended on the degree of soil contamination.[15,26,27] So, in this study the contamination rate of soil samples collected from different parts of Isfahan

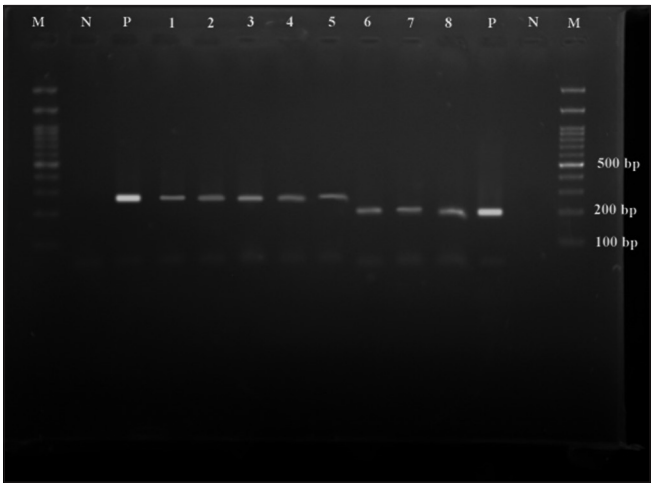

Figure 3: Results of PCR experiment following gel electrophoresis indicating 260bp band for *T. canis* and 204bp for *T. cati*, (M = Marker, *P* = positive control, N = negative control for *T. canis* and *cati*), and Lines 1, 2, 3, 4, 5 with a length of 260bp indicate *T. canis* samples and lines 6, 7, 8 with a length of 204bp indicate *T. cati* samples

Table 2: Prevalence of *Toxocara spp* eggs in soil samples in Isfahan province of Iran based on molecular method

Location	Total Sample (No)	Positive (No)	Percent (%)	T.*cati* (No %)	*T.canis* (No %)	Mix (No %)
Ardestan	60	9	15	3 (5)	5 (8.33)	1 (1.66)
Semirum	60	21	35	5 (8.33)	11 (18.33)	5 (8.33)
Fereydun Shahr	60	23	38	6 (10)	14 (23.33)	3 (5)
Naein	60	8	13.3	4 (6.66)	4 (6.66)	0
Esfahan	115	26	22.60	13 (11.30)	10 (8.69)	3 (2.60)
Total	355	87	24.50	31 (8.73)	44 (12.39)	12 (3.38)

Table 3: Prevalence of *Toxocara spp* eggs in soil samples in Isfahan province of Iran based on location of sample collection

Location	Total Sample	Positive (No)	Percent (%)	*T. cati* (No %)	*T. canis* (No %) s	Mix (No %)
Home	70	14	20	9 (12.85)	4 (5.71)	1 (1.42)
Busy area	40	7	17.5	3 (7.5)	4 (10)	0
Park	45	16	35.5	3 (6.66)	8 (17.77)	5 (11.11)
Play ground	90	29	32.22	10 (11.11)	16 (17.77)	3 (3.3)
Dormitory	15	2	13.3	2 (13.33)	0	0
Slaughterhouse	95	19	20	4 (4.21)	12 (12.63)	3 (3.15)
Total	355	87	24.50	31 (8.73)	44 (12.39)	12 (3.38)

Table 4: Prevalence of *Toxocara spp* eggs in soil samples in Isfahan province of Iran based on climate condition

Climatic conditions	Total Sample	Positive (No)	Percent	*T. cati* (No %)	*T. canis* (No %)	Mix (No %)
Warm-dry	120	17	28.3	7 (11.66)	9 (14.99)	1 (1.66)
Cold-dry	60	21	35	5 (8.33)	11 (18.33)	5 (8.33)
Wet-cold	60	23	38	6 (10)	14 (23.33)	3 (5)
Mild	115	26	22.60	13 (11.30)	10 (8.69)	3 (2.60)
Total	355	87	24.50	31 (8.73)	44 (12.39)	12 (3.38)

province has been investigated using both microscopic and molecular methods.

In different investigations in Iran, contamination rates of 6.3%, 22.2%, and 38.7% were reported for soil samples collected from Urmia, Khorramabad, and Tehran, respectively.[27-29] While in our work contamination rates of 21.69% and 24.5% were achieved using parasitological and molecular methods, respectively.

Soil contamination rates may vary in different regions due to climate and presence of stray animals.[30] In this regard, contamination rates of 9.7%, 6.7%, 28.1%, 11.57%, 55%, 14.03%, 11.87% has been reported for North America, Latin America, Europe, Asia, the Middle East, Australia and Turkey, respectively.[31] These different results are due to several different factors such as climate, poverty, social status, type of soil, and number of stray dogs and cats in these areas.[32] In a work in Turkey, soil contamination with *Toxocara* spp in Fenced parks and Fenceless parks were different.[33] Differential diagnosis of *Toxocara spp*. is very important in epidemiological studies. Microscopic examination is not suitable for distinguishing between two species of *Toxocara*, especially for soil samples. Molecular methods are usually considered as an accurate method for differentiation of *Toxocara* species. Most studies have shown that dog's *Toxocara* is the predominant species, however, other studies have also mentioned the cat's species in some areas is the dominant strain. Our work is the first report of soil contamination with *Toxocara spp* eggs in dogs and cats in Isfahan province in central Iran. According to our results in Fereydun Shahr and Semirum counties, higher contamination rates were seen. So, in these counties *Toxocara* infection may easily happen in human. Control measures, especially control of stray dogs and cats should be applied by health authorities in these two regions.

Conclusion

According to results of this work, *Toxocara* parasite eggs exist in the soil of public areas of Isfahan province. The contamination rate was higher in Fereydun Shahr and Semirum counties.

Ethics approval

This work was approved by Isfahan University of medical sciences research ethical committee with code number of IR.MUI.MED.REC.1400.529

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Conflicts of interest

There are no conflicts of interest.

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