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

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 in 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 GTTGTTGG-3’) and reverse primer (NC2: 5’- TGGCCATGATTCCCTATTC-3’) and for T. cati forward primer (NC5: 5’-CTTCTGGTGCAATTCTTT CGC-3’) and reverse primer (NC2: 5’- CCAAGCAACAA CAAAATAGCG-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       |
| Isfahan          | 115               | 24            | 20.86       |
| Total            | 355               | 77            | 21.69       |

![Figure 1: Toxocara parasite egg observed in soil samples collected from Isfahan province of Iran](image)
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.

### 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. So, in this study the contamination rate of soil samples collected from different parts of Isfahan.

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#### 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 %) | 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)  |
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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**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)  |

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**Conflicts of interest**

There are no conflicts of interest.

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