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Original Article

The Study of Soil Contamination by *Toxocara* spp. Eggs in Different Areas of Chaharmahal and Bakhtiari Province, Southwest Iran

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Abstract

Background and aims: Toxocariasis caused by the larvae of *Toxocara* spp. is a zoonotic infection with global distribution that is considered an important health problem in the humans. The soil is regarded as the main source of the transmission of *Toxocara* infection to susceptible hosts. This study investigated the existence of *Toxocara spp*. ova in rural and urban public areas of Chaharmahal and Bakhtiari province.

Methods: A total of 180 soil samples were randomly collected from 74 public places from urban and rural areas of nine counties of Chaharmahal and Bakhtiari province during May to September, 2017. The samples were examined for *Toxocara* spp. eggs by the modified sucrose flotation method.

Results: The contamination rate of the soil samples with *Toxocara* spp. ova varied within 0%-18.1% in different counties. Overall, 9 (5%) out of 180 examined soil samples were found positive for *Toxocara* spp. ova. Of nine investigated districts, *Toxocara* spp. ova were observed in four counties and the highest rate of soil contamination was found in Farsan county with 18.1%. Finally, the prevalence of *Toxocara* spp. eggs was more in the rural areas (6.7%) compared to the urban ones (3.3%).

Conclusion: This study was the first investigation regarding the contamination of *Toxocara* spp. eggs in the soil of public places in rural and urban areas of Chaharmahal and Bakhtiari province. According to the results, the rate of soil contamination in this region was lower compared with the other parts of Iran. Therefore, a further study is required to determine factors that may be involved in the distribution of *Toxocara* spp. in different areas of this province.

Keywords: *Toxocara* egg, Floatation, Soil, Chaharmahal and Bakhtiari province, Iran

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Introduction

Toxocariasis is a widespread serious zoonotic helminth disease caused by the larvae of the parasitic ascarid nematodes of the genus *Toxocara* of domestic and wild carnivorous in both developed and developing countries worldwide. *Toxocara canis* and *Toxocara cati* in the Toxocaridae family are the only two species that are known to cause human toxocariasis or *Toxocara* larva migrans.[1,2] In the life cycle of *Toxocara* spp., adult worms live within the lumen of the small intestine of such domestic and wild carnivores as dogs and cats as the definitive hosts of *Toxocara canis* and *Toxocara cati*, respectively, shedding the helminth eggs in the nature by the defecation. Ingesting the viable and embryonated eggs from contaminated sources (e.g., soil, raw vegetables, and the like) can transfer the infection to the humans. In addition, human can be infected by the ingestion undercooked infected meat of paratenic hosts such as chickens, pigs, and ruminants.[3] Concerning the completion of the life cycle, the human is the aberrant host. The infective larvae hatch after the ingestion of the embryonated eggs, but the juvenile stages fail to develop to mature adult worms.[4,5] They move toward other organs and tissues. Although their destination is generally the liver and the lungs, they may be also located in the kidney, heart, retina, and the central nervous system. The migration of the larvae to the viscera leads to a condition called visceral larva migrans. Serious ocular damage, known as ocular larva migrans, can also occur when the larvae arrive at the retina.[6-8] The associated symptoms and complications of this socioeconomic important zoonotic

disease include vomiting, anorexia, fever, recurrent abdominal pain, pulmonary problems, allergies, and neurological disorders.[1,2,9] Human *Toxocara* infection has a worldwide distribution. Further, the soil functions as the main source of the transmission of *Toxocara* infection and can cause significant health problems for the humans.[10,11] Based on different reports, the prevalence of *Toxocara* spp. eggs in the soil of various countries and regions varies from 2% to 88% based on the sanitary behaviours of residents and pet owners, the type and number of dogs and cats, the access of stray animals to public places, climatic conditions (e.g., temperature and humidity), geographical situations, the sample size, and the techniques and methods for the egg detection in the soil.[12] However, it was estimated that a fifth of public areas in the world are contaminated with *Toxocara* eggs.[13] The increasing population of dogs and cats infected with the parasites in public areas such as public parks and their easy access to this area has caused a growing prevalence rate of the infection among the children. This group is at a higher risk of acquiring the infection with the parasite when they eat the soil contaminated with eggs or put objects contaminated with eggs in their mouths.[9,14] In recent years, several studies have focused on investigating the *Toxocara* eggs in the soil of the public parks in Iran. According to several studies, the prevalence of *Toxocara* eggs differs from 5% to 63% in different areas.[15-20] Chaharmahal and Bakhtiari province, located in the southwest of Iran, is one of the important regions for animal husbandry. A recent serological study demonstrated a 2% prevalence of toxocariasis in 2-14 year-old children in this province.[21] With regard to the importance of this zoonotic disease, this cross-sectional study was the first one, to the best of our knowledge, to evaluate the prevalence of the contamination of *Toxocara* eggs in the soil of the public parks and gardens across the entire urban and rural areas of Chaharmahal and Bakhtiari province.

Materials and Methods

Field Study Area

Chaharmahal and Bakhtiari province (16532 square km), one of the mountainous parts of Iran's central plateau, is situated between 31° 09' and 32° 48' north latitude, as well as between 49° 28' and 51° 25' east longitude, with a population of about 895263 persons. In addition, this province has various climates in different areas ranging from cold, snowy, and rainy weather, especially in the fall and winter, to hot weather in the summer. The jobs of many people in this province include animal husbandry, farming, and gardening. At the time of the study, this province had nine counties including Shahrekord, Farsan, Boroojen, Lordegan, Ardal, Kiar, Ben, Saman, and Kohrang. Shahrekord, the capital of this province, is the highest capital city of Iran at 2,066 m above sea level.

Sample Size

A total number of 180 soil samples were randomly collected from 74 public places from 9 counties of Chaharmahal and Bakhtiari province during May to September 2017. Each sample consisted of 150 g of soil which was collected from an area of 20 cm^2 and cm^3 depth. The samples were distinctly carried to the laboratory of the Parasitology Department of Shahrekord University of Medical Sciences.

Parasitological Procedures

The samples were examined for *Toxocara* spp. by the floatation method using saturated sucrose (1.2 g/cm^3) based on the method by Maraghi et al with several modifications.[16] After collecting the samples, the soil was dried at room temperature for 2-3 days. Further, after sieving by 150 µm mesh, 20 g of each dried soil samples was added to 70 mL distilled water containing some droplets of detergent and then mixed well. The mixture was filtered by three layers of mesh material, then centrifuged at 2000 rpm for 5 minutes. Next, the supernatant was discarded and the precipitation was re-suspended in the normal saline, followed by repeating the washing process. Furthermore, the supernatant was discarded and the saturated sucrose solution was added gradually to the precipitate by shaking the tube. Moreover, the suspension was centrifuged at 1500 rpm for 15 minutes. Then, the sucrose solution was added to fill the top of the tube. Additionally, the coverslip was placed on the tube in touch with the sucrose, kept in the rack for 45 minutes, and then was removed and placed on a glass slide and studied under the light microscope.

Statistical Analyses

SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA) was used for all descriptive statistics. The chi-square test was applied to access the univariate association between independent variables and the outcome. In this survey, $P<0.05$ was considered statistically significant.

Results

Out of 180 examined soil samples for *Toxocara* species eggs, 9 (5%, 95% CI: 2.2-8.9) cases were found positive for *Toxocara* spp. eggs (Figure 1). The contamination of samples with *Toxocara* spp. eggs was within the range of 0%-18.1% (95% CI: 2.2–9.4) in different counties. These results did not show any statistical association between the infected soil and the county ($P=0.075$). The prevalence of *Toxocara* spp. egg in the rural areas (6.7%, six samples) was more than that of the urban areas (3.3%, three samples; 95% CI: -0.099–0.030), the details of which are shown in Table 1 and Figure 2. Based on the results, the prevalence of *Toxocara* egg in the contaminated soil was not significantly related to the place of sampling ($P=0.289$).

Discussion

Toxocara canis and *T. cati* are common gastrointestinal

Table 1. The Prevalence of *Toxocara* spp. Egg in the Soil of Different Regions of Chaharmahal and Bakhtiari Province, Southwest Iran

Area (County)	Number of Samples (%)	Place of Samples		Number of Infected Samples (%)
		Urban Areas (%)	Rural Areas (%)	
Shahrekord	18 (10)	8 (44.4)	10 (55.6)	NS
Farsan	22 (12.2)	10 (45.5)	12 (54.5)	4 (18.1)
Boroojen	30 (16.7)	20 (66.7)	10 (33.3)	3 (10)
Lordegan	20 (11.1)	8 (40)	12 (60)	1(5)
Ardal	20 (11.1)	5 (25)	15 (75)	NS
Kiyar	10 (5.6)	0 (0)	10 (100)	NS
Ben	20 (11.1)	10 (50)	10 (50)	NS
Saman	20 (11.1)	10 (50)	10 (50)	NS
Kohrang	20 (11.1)	10 (50)	10 (50)	1 (5)
Total	180 (100)	91 (50.6)	89 (49.4)	9 (5)

NS: No seen

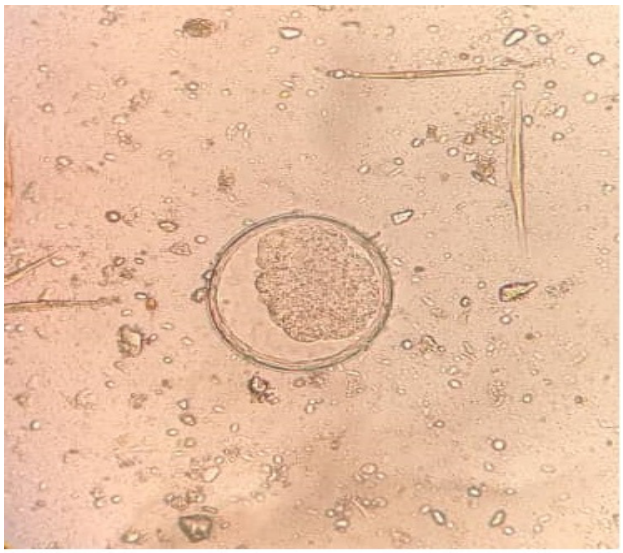

Figure 1. *Toxocara* spp. Egg Isolated From the Soil of Public Places in Chaharmahal and Bakhtiari Province (400×).

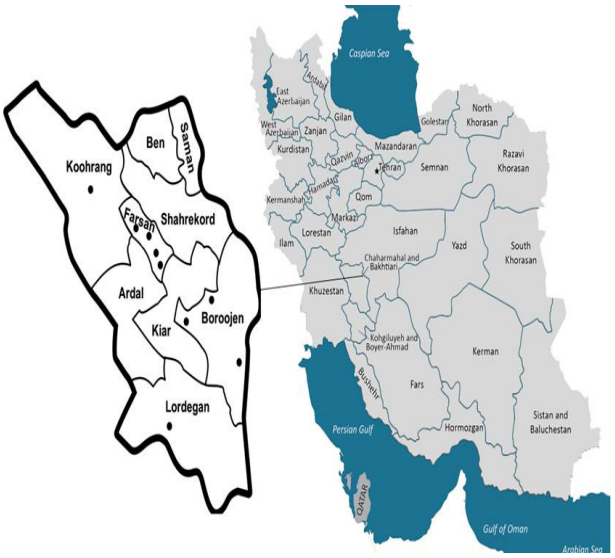

Figure. 2. Geographical Location of Chaharmahal and Bakhtiari Province and the Contaminated Spots.

parasites of most wild and domestic carnivores such as dogs and cats, respectively. It is estimated that there are over 600 million cats and 900 million dogs as stray and pet animals around the world, many of which may be infected with different parasites including gastrointestinal parasites. Therefore, these animals can defecate million tons of infected feces with discharge helminth eggs and protozoan cysts with zoonotic importance into the public environments every year. Despite efficient public health and hygiene promotion in different societies, many people such as gardeners, farmers, construction workers, and municipality staff, as well as toddlers and young children, may be exposed to contaminated soil based on occupational groups and behavioral characteristics such as playing in the parks as high-risk groups for toxocariasis.[22] The occurrence of *Toxocara* eggs in public parks is a matter of concern for public health.[6] The children with pica are at a higher risk of consuming infective eggs from the soil than those not exhibiting this habit[4].

In this study, the soil contamination by *Toxocara* spp. eggs was investigated for the first time in different areas of Chaharmahal and Bakhtiari province, Iran. Based on our findings, the contamination rate of *Toxocara* eggs in the collected soil samples was 5% by the parasitological method. In addition, from nine investigated counties for the *Toxocara* egg, only four cases found contamination with the maximum rate (18.1%) in Farsan county. Nevertheless, different studies indicated that the contamination rate is variable in different counties. Several studies were carried out to verify the contamination of the soils in different regions of Iran. Based on a systematic review and meta-analysis of 14 studies carried out in different areas of Iran, the weighted overall prevalence of *Toxocara* spp. was 16% in 3031 soil samples. Further, Tehran (38.7%) and Qazvin (2.3%) provinces had the highest and lowest prevalence rates, respectively.[3] These studies are often conducted in

urban areas and limited to a narrow geographic range. The results of some of these studies are in line with our results, while those of some of the other studies contradict the results of this study. For example, the findings of studies conducted in Qazvin-Sari (3.73% in the North of Iran),[23] (5.8% in the north-west of Iran),[17] Shiraz (6.3% in the south of Iran),[24] and Urmia (7.8% in Northwest Iran)[19] are consistent with our results. However, the rate of contamination in Khorramabad (22.2% in the west of Iran),[18] Ahvaz (28.4% in the southwest of Iran),[25] Abadan (29.2% in the southwest of Iran),[16] Karaj (36.4% in the north-central of Iran),[26] and Tehran (38.7% in the north of Iran)[27] is very different from the obtained result in this regard in our study. In the present study, the prevalence of the *Toxocara* egg in the soil of the rural area was more compared to the urban area, which may be due to the existence of stray and shepherd dogs in and around the villages. Despite the results of this study, the frequency of *Toxocara* egg in the soil was higher in the cities in some investigations. Mizgajska-Wiktor et al reported that the level of the soil contamination with *Toxocara* eggs in the cities was higher compared to villages and small towns in Poland, which was associated with the population density of the owner's cats and dogs as pet animals.[28] The differences between the observations in the rate of *Toxocara* spp. egg across diverse regions may be due to the number of cats and dogs in each area, as well as the variations in environmental conditions such as sunlight, temperature, humidity, and the geographical location of these areas. One of the reasons for the difference in contamination rates can be due to the number of samples in relation to the different regions. In other words, taking more samples in a geographic area increases the chance of finding positive samples. Some studies regarding the prevalence of *Toxocara* eggs in the soil samples in Europe and Asia reported values of 3.2%-64% and 5.7-95%.[29-32] Furthermore, the contamination rate of *Toxocara* eggs in North and South America was reported as 0.3%-39% and 0.3-79.4%.[33,34] The differences in the social and religious circumstances of Iran from other countries can be considered as causes of the discrepancies between the rates of contamination.

In recent years, few serological studies have been conducted in Chaharmahal and Bakhtiari province regarding the evaluation of human toxocariasis. In this regard, the results of a study on 2-14-year-old children demonstrated that the prevalence of toxocariasis was 2% of the investigated population in Chaharmahal and Bakhtiari.[21] In another study, the seroprevalence of toxocariasis in children under ten years old was 50.9% in the serum samples in Chaharmahal and Bakhtiari tribes by using the ELISA method.[35] Finally, it should be noted that the difference in the results of this study from those of other studies could be attributed to the number of the collected soil samples, the depth of the soil, and the diagnostic method. Meanwhile, the number of stray dogs and cats in public places can affect the prevalence of *Toxocara* spp. eggs. Furthermore, climatic differences, rainfall, and temperature should be considered in different areas.

Conclusion

To the best of our knowledge, this study was the first investigation conducted on *Toxocara* spp. soil contamination in the public rural and urban areas of Chaharmahal and Bakhtiari province. According to the results, the rate of soil contamination in this region was lower compared with the other parts of Iran. Further studies are required to determine the factors that may be involved in the distribution of *Toxocara* spp. in different areas of this province.

Ethical Approval

This survey was approved by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.REC.1396.235).

Conflict of Interest Disclosures

We declare that we have no conflicts of interest.

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Author Contributions

GS and KMN designed the experiments. In addition, GS, RA, and MK performed the experiments. Further, KMN and SM analyzed the data, and finally, GS and RA wrote the paper.

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