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Original paper

High contamination of soil with *Toxocara* spp. eggs in the north of Iran

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ABSTRACT. Toxocarosis is an important emerging zoonotic disease, which is caused by larvae of *Toxocara canis* and *Toxocara cati*, the most widely distributed nematode parasites of dogs and cats. The soil, where *Toxocara* eggs become infective, is the natural source of infection. The aim of this work was to determine the levels of soil contamination with *Toxocara* spp. eggs in Sari district, north of Iran, in order to estimate the risk of infection of the population living in this region. A total of 141 soil samples were collected from 12 parks, 12 primary schools and 45 backyards from three regions. Soil samples were investigated for the presence of *Toxocara* eggs by flotation technique using sucrose solution and examined under light microscope using 10× and 40× objective. Out of 141 soil samples, 67 (47.5%) were found to contain *Toxocara* spp. eggs. The contamination rate in public parks, primary schools and backyards were observed 50%, 58.3% and 33.3%, respectively. The comparison of soil contamination rate from different areas, public parks, primary schools and backyards, was statistically significant (χ^2=6.00, d.f.=2 and *P*=0.049). The public parks, backyards and primary schools, which are main places that children play, are suitable for defecation of dogs and cats. The knowledge of the soil contamination with *Toxocara* eggs indicates the need to take an appropriate and effective measures to prevent the infection.

Keywords: zoonotic infectious diseases, *Toxocara* eggs, soil contamination, public health, soil pollutants

Introduction

Toxocara cati and *Toxocara canis* are common nematodes living in the intestine of dogs and cats. Soil is the natural source of infection, where *Toxocara* eggs become infective and withstand for one to three years in the environment [1]. Human toxocarosis is caused by the ingestion of food and soil contaminated with embryonated eggs of *Toxocara* spp. [2]. This disease is an important emerging zoonotic disease, and should be considered as a serious public health problem. According to the different symptoms of disease, four syndromes are recognized; visceral larva migrans (VLM) with allergic asthma, eczema and abscess in the organs; ocular larva migrans (OLM) without the eosinophilia, but with *Toxocara* larvae invading the eye; neurological toxocarosis with different manifestations such as meningo-encephalitis, myelitis, optic neuritis, cerebral vacuities and epilepsy; and covert toxocarosis with non-specific symptoms [3]. In public spaces such as

parks and playgrounds, where animals freely have access to, they can shed *Toxocara* spp. eggs with their faeces to the environment and thus risk of infection raises for the population [4,5]. Direct contact with cats and dogs does not consider as a potential risk because the eggs excreted with the definitive host's faeces need a period of about 4–6 weeks to become infective, therefore, contact with soil contaminated with embryonated *Toxocara* eggs is a potential source of infection [6]. Children are at the risk group for the infection because they have immature immune system and often play in the places such as parks, playgrounds and backyards, where have been contaminated with *Toxocara* spp. eggs [7,8] Moreover, consuming soil, a specific type of pica that increases the risk of *Toxocara* infection, is common among children [4] There are several reports in Iran and other countries that show widespread soil contamination of the public parks, playgrounds, primary schools and other places with the eggs of *Toxocara* spp. [9,10]. The aim of this work was to determine the levels of soil contamination with *Toxocara* spp. eggs in Sari district, north of Iran in order to estimate the risk of infection of the population living in this region.

Materials and Methods

Survey area

Sari is located in the center of Mazandaran province, north of Iran. This city is situated between the parallels 35°58′ and 36°50′ of the north latitude and between 52°56′ and 53°59′ of the eastern longitude. Its' population is around 500000 and Sari has a temperate climate with rainfall occurrence in all seasons of the year. The mean yearly relative humidity is 85.8% and an average temperature of 17°C. Sari is divided into three regions. Region one consists mainly of small parks and tall buildings. The inhabitants mainly have good hygiene and high income. Region two has comparatively larger areas of green parks. The inhabitants mainly have average hygiene and income. Region three consists of large open areas and part of this region situated on the suburban areas of the Sari. The inhabitants mainly have poor hygiene and low income. Stray dogs and cats are many in this area.

Sampling/parasitological procedure

During the period from September to February, a total of 141 soil samples were collected from 12 public parks, 12 primary schools and 45 backyards of Sari. In each of the three above-mentioned regions, the samples were collected from four parks and four primary schools. The samples were collected from four different parts of each park and primary school, including northern, eastern, western and southern and one sample from each backyard. We collected samples of loose soil, mainly from the flowerbeds. Each sample contained approximately 200 grams of soil from a depth of 2–8 cm, because *Toxocara* spp. eggs are more abundant at this depth [11].

By using 150 μm mesh sieves, 2 g of each sample was isolated and transferred into the tubes, and then 10 ml Tween-80 with 0.05% of concentration added to the mixture and shook vigorously then centrifuged at 1500 rpm for 5 minutes. After discarding the supernatant, the test tube containing the sediments was filled with approximately 1 cm from the top with sucrose solution (1.2 g/cm^3) and the sediment was suspended. In the next step, the tubes were centrifuged for 15 min at 1500 rpm and finally the tubes were filled to the top with sucrose solution and a cover slip placed on the tube for 30 min and then examined under a light microscope using 10× and 40× objective.

Results

A total of 141 soil samples were examined, which 67 (47.5%) were found to contain *Toxocara* eggs (Tab. 1). *Toxocara* eggs observed in 11 (91.7%) of 12 studied parks and 12 (100%) of 12 studied primary schools. Twenty four (50%) out of 48 samples from public parks, 28 (58.3%) out of 48 samples from primary schools and 15 (33.3%) out of 45 samples examined from backyards were found to be positive about *Toxocara* eggs. By Chi-square test, the relationship between contamination rate in public parks, primary-schools and backyards were evaluated and the results showed that the frequency of soil contamination between studied sites was significantly different (χ^2=6.00, d.f.=2 and *P*=0.049).

Prevalence and number of positive soil samples for *Toxocara* eggs from different regions of the city are shown in table 1. There was significant difference between contamination rate and different regions of Sari (χ^2=7.56, d.f.=2 and *P*=0.023). Region three was found to be highly contaminated (59.6%) comparing to the other examined sites and the lowest rate of contamination was observed in region one (31.9%) (Tab. 1).

Table 1. Soil contamination to *Toxocara* eggs in different parts of Sari

Regions	Public parks		Primary schools		Backyards		Total	
	Sample no	Positive n (%)	Sample no	Positive n (%)	Sample no	Positive n (%)	Sample no	Positive n (%)
1	16	5 (35.3)	16	7 (43.7)	15	3 (20)	47	15 (31.9)
2	16	9 (56.2)	16	9 (56.3)	15	6 (40)	47	24 (51.1)
3	16	10 (62.5)	16	12 (75)	15	6 (40)	47	28 (59.6)
Total	48	24 (50)	48	28 (58.3)	45	15 (33.3)	141	67 (47.5)

Discussion

Infections with soil transmitted helminths are concern in both developing and developed countries [5]. Comparing to the previous studies, 47.5% of soil contamination with the parasite's egg in Sari is the highest rate to date in Iran (Tab. 2). Similar surveys were performed in other countries and contamination rates were highly varied from 0.7% in Argentina to 97.5% in Greece [12,13]. According to our results, soil contamination of public parks (50%) and primary schools (58.3%) with *Toxocara* eggs are fairly high in Sari. Regarding to studies carried out in different regions of Iran, the highest rate of soil contamination of parks reported was 38.3%, which is lower than our results [14]. Also the contamination rate observed is higher in Sari than Poland 35.5% [15], Russia 23.3% [16], Greece 17.2% [17], Egypt 12.5% [18] and Thailand 12% [19]. In Europe, public places, including playgrounds and parks considered as the main area of risk of human exposure to *Toxocara* eggs [20]. The green spaces of the parks, where mainly children play in, are suitable for defecation of dogs and cats. These contaminated faeces can transmit *Toxocara* eggs to the humans, especially to the children [21]. Seroprevalence of toxocarosis in schoolchildren in Sari was reported at 25% [22]. In the United States, seropositive rate of toxocarosis have been reported at 4.3%–5.9% among children ≥6 years old [23], but in children aged 5–15 years was 1.39% in center of Iran [24]. Other studies report seropositivity rates of toxocarosis 15.5% in Brazil, 8% in Italy, and 4.2 in Denmark [25–27]. According to studies mentioned above, the high rate of soil contamination with *Toxocara* in primary schools of Sari should be considered as a serious problem.

Soil transmitted toxocarosis in children has been reported 2–80% [28]. Poverty, poor hygiene, lack of education, untreated and uncontrolled definitive host populations and geophagia will lead to *Toxocara* infection in children [29,30]. In our study the contamination rate of backyards was lower than parks and primary schools. Few people in Sari keep dogs in their house and their backyard is mostly surrounded by walls. So the most possible explanation for contamination of backyards with *Toxocara* eggs is maybe related to stray cats.

In Sari, the prevalence rate of *T. canis* and *T. cati* in stray dogs and cats have been reported 60% and 44%, respectively, which seems very high [31,32]. In Sari, climatic conditions are suitable for the spread and survival of *Toxocara* eggs. Temperate climate can enhance the embryonation of *Toxocara* eggs in the soil and therefore, increases potential transmission to humans. In similar studies, the impact of climate on this issue has been identified [6,33,34]. According to the result of this study, there was a significant difference in the contamination rate among the different regions. Region three with 59.6% has the highest contamination rate in Sari. Many studies showed an association between socioeconomic status and prevalence of parasitic infections as an important factor [35,36]. Region three located in the margin of Sari with poor hygienic status and many stray dogs and cats, therefore, the high contamination rate in this region is explainable.

The public parks, backyards and primary schools of Sari, north of Iran are highly contaminated by *Toxocara* spp. eggs. Preventive measures seems to be necessary in this region, which could include health education of the society, improving sanitation conditions, especially for underprivileged peoples, and control of stray dogs and cats near play areas of children.

Table 2. Studies carried out on soil contaminated with *Toxocara* eggs in Iran after 2010

City name/location of Iran	Location of study	Samples No	Prevalence (%)	Reference
Sari/north of Iran	Public parks, primary schools, backyards	141	47.5	Present study
Tehran/Central of Iran	Public places, playground	150	38.7	[14]
Karaj/west of Tehran, Iran	Public parks	200	36.4	[1]
Tabriz/northwest of Iran	Public parks	540	34.44	[37]
Abadan/southwest of Iran	Public parks	291	29.2	[33]
Isfahan/Central of Iran	Public parks	140	28.6	[8]
Ahvaz/southwest of Iran	Public parks	260	28.4	[38]
Khorram Abad/west of Iran	Public parks	285	22.2	[39]
Khorramshahr/southwest of Iran	Public parks	150	18	[34]
Kermanshah/west of Iran	Public parks	150	18	[40]
Kermanshah/west of Iran	Public Places	126	13.5	[41]
Ilam/west of Iran	public places	130	13	[2]
Tehran Province /Central of Iran	Public parks	1132	11.39	[42]
Khaf/northeast of Iran	Public parks	145	10.3	[43]
Tehran/central of Iran	Public parks	600	10	[44]
Tabriz/northwest of Iran	Public parks	300	9.3	[45]
Mashhad/northeast of Iran	Public parks	195	7.7	[43]
Ardabil/northwest of Iran	public places	200	7	[46]
Chaharmahal and Bakhtiari/ Central of Iran	public places	180	5	[47]
Jahrom/South of Iran	public parks, elementary schools and kindergartens	100	4	[48]
Qazvin/northwest of Iran	Public parks	95	3.15	[49]

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