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Prevalence of *Toxocara* Species Eggs in the Soil of Public Parks in Hamedan City, Western Iran

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Abstract

Background: Toxocariasis is one of the most commonly reported zoonotic helminth infections in the world which is caused by the parasitic roundworms of dogs or cats. This survey aimed to provide data on the contamination of the soil of public parks by *Toxocara* spp. eggs in Hamedan city, capital of Hamedan Province in the west of Iran.

Methods: During April and May 2019, a total of 120 soil samples were collected from 20 public parks (4 to 10 soil samples from each park) from all geographical areas of the city. For detection of eggs, soil samples were dried overnight at room temperature and passed through a 150 μ m mesh sieve. After performing Sheather's flotation technique using 2 g of powdery soil, light microscopic examinations were carried out.

Results: Microscopic evaluation of specimens revealed that 14 samples out of 120 (11.67%) collected from 10 public parks out of 20 in Hamedan (50.0%) were contaminated with *Toxocara* spp. eggs. Various developmental stages of *Toxocara* spp. eggs including (1) non-developed, (2) 2-cell to tadpole stage, and (3) larvated eggs were observed. Furthermore, in two public parks, *Trichuris* spp. eggs were found in the soils.

Conclusions: This study provides the first information about the contamination of public areas in Hamedan Orovince and suggests that public awareness about this fact is needed. Education of citizens especially parents with children, who have commonly mouthing behavior, is recommended. Further molecular-based studies for the identification of parasite species in the province are suggested. All of the published literature about contamination of the soils of public areas in Iran with *Toxocara* eggs until July 2020 were also reviewed.

Keywords: Toxocariasis, Zoonosis, Soil-transmitted helminths, Public area, Hamedan, Iran

Background

Toxocariasis caused by the larval stage of Toxocara canis and Toxocara cati in humans is a neglected infection and one of the most widespread zoonotic disease with public health and socioeconomic importance worldwide (1,2). Upon ingestion of embryonated eggs, the larvae are released from the eggs in the small intestine, penetrate the intestinal wall, and migrate to liver, lungs, and other organ systems causing visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT), and covert or common toxocariasis (CT) (3). Visceral toxocariasis is a febrile disease particularly affecting children in their first decade of life due to eating soil, mouthing of objects, playing in sandboxes or playgrounds contaminated with faeces of dogs and cats, and/or their poor understanding of hygiene (4). *Toxocara* infection is caused mainly by the accidental ingestion of the infective eggs of *Toxocara* spp. through contaminated soil, water, fruit, or vegetables. It can also be caused by the ingestion of the encapsulated third-stage larvae present in raw/undercooked meat and/

or organs from paratenic or transport hosts, such as rabbits, ruminants, and poultry (5). Another mode of transmission is reported to be human contact with dogs or cats, as embryonated eggs could attach to their fur (6). On a worldwide scale, 20%-40% of dogs and cats with parasitic worm burdens have T. canis or T. cati (7), and national surveys on humans have reported the prevalence of antibodies against *T. canis* ranging from 1.6 to 81% in different countries (1). In Iran, Toxocara and Toxascaris spp. are among the most frequently observed parasites with a pooled prevalence of 32.6% in dogs, 24.2% in cats, 69.4% in red foxes and 23.3% in golden jackals, and it has been estimated that 9.3% of the general population have antibodies against *T. canis* (8). The environmental contamination with Toxocara eggs, because of the infected carnivores defecating in playgrounds, sandpits, gardens, parks, and beaches, is one of the major causes of human toxocariasis (7). Several studies have investigated contamination of soil of public areas in different regions of the world and the estimated prevalence rates in the

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different WHO regions ranged from 13% to 35% (9). According to the published reports, 18% of soil samples from the Middle East and North Africa were contaminated with *Toxocara* spp. (range: 11%–24%) (9). In Iran, a recent meta-analysis article reported that 16% of soil samples from public areas are contaminated with the eggs of *Toxocara* spp. (10). Considering (i) notable prevalence of toxocariasis is in both stray and owned dogs (11, 12) and cats (first author's personal experience), (ii) presence of antibodies against *T. canis* in blood sera of 8.8% of children in the urban and rural areas of Hamedan (13), and (iii) the public health importance of contamination of parks with *Toxocara* spp. eggs, this study aimed to examine soil samples from different public parks of the city for the presence of *Toxocara* eggs.

Methods Study Area

Hamedan city (34° 48′ 0″ N, 48° 31′ 12″ E) is the capital of Hamedan province in the west of Iran with around 600 000 inhabitants. It has a cold semi-arid climate with an annual rainfall of 318 mm and an annual average temperature of 11.3°C. In the city, there are 130 public parks under different categories such as pocket, neighborhood, regional, district, and city parks with various sizes distributed in four districts (14).

During April and May 2019, a total of 120 soil samples were collected from 20 district and city parks with an overall area of 551 241 m². Public parks namely Aramgah-e Bu-Ali Sina, Mardom (also known as Luna), Abbas Abad, Aramgah-e Baba Taher, Eram, Namaz, Bagh-e Irani, Moadarres, Etemadieh, Koodak, Shahrvand, Parvaz, Narges, Maryam, Madar, Shokri Pour, Banovan, Bagh-e-Nazari, Chamran and Banafsheh (Figure 1) are distributed in all four city districts. These parks are crowded all year round, along with children's playgrounds and camping sites.

Sampling and Detection of Helminths Eggs

From each park, four to ten soil samples were taken based on the area of the parks i.e., separate samples were collected from every 50 steps. Soil samples of approximately 200 g were collected at a depth of 3 cm, stored in plastic bags, and taken to the laboratory. For detection of ova, soil samples were dried overnight at room temperature and passed through a 150 µm mesh sieve. Exactly 2 g of powdery soil was subjected to a normal sucrose flotation method (15). Light microscopic examinations were carried out by trained personnel and photomicrographs were taken from positive samples. Eggs were counted and classified according to their developmental stage i.e., non-developed, 2-cell to tadpole stage, and larvated eggs (16).

Results

Fourteen out of 120 samples (11.67%) collected from 10 public parks out of 20 in Hamedan (50.0%) were contaminated with *Toxocara* spp. eggs.

The ratio of the number of positive specimens to the total number of collected soil samples from each park ranged from 12.5% to 50.0%.

The eggs were spherical to subspherical, measuring approximately 75–85 μ m in diameter with a thick shell, an alveolar external layer (thimble-like), and a smooth internal layer as already described (7). All three developmental stages of *Toxocara* spp. eggs i.e., non-developed (n=2; 14.30%), developing stages (n=6; 42.85%), and larvated eggs (n=6; 42.85%) were observed (Figure 2). Furthermore, in two public parks, *Trichuris* spp. eggs were found in the soils.

Discussion

In this study, 11.67% of soil samples were contaminated with eggs of *Toxocara* spp. Several studies in different cities of Iran have reported contamination rates ranging between 4 and 63.3% in public areas. All of the studies

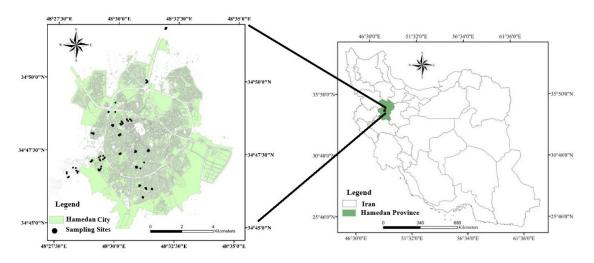


Figure 1. Location of Sampled Sites.

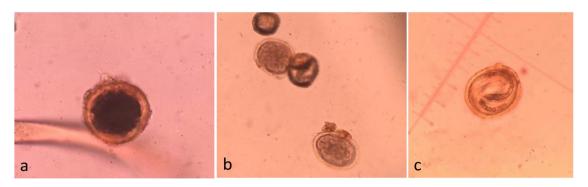


Figure 2. Different Stages of Toxocara spp. Eggs in Soil Samples; (a) Non-developed Egg, (b) Dividing Egg, (c) Larvated Egg.

except two are based on light microscopy examination of soil specimens (Table 1). Furthermore, in the present study, 50.0% of the public parks were found to be contaminated with Toxocara spp. eggs. In similar studies different rates were reported i.e., 80% in Piranshahr (32), 77.77% in Zanjan (42), 76.7% and 14.7% in Tabriz (27,29), 75% in Isfahan (37), 61.2% in Abadan (24), 50% in Ilam (33), 36.4% in Karaj (39), 34.1% in Ardabil (28), 31.25% in Larestan (21), 26.66% in Arak (38), 23.2% in Tehran (36), 22.2% in Khorram Abad (15), 15% in Shiraz (18), and 3.9% in Urmia (26). It has been shown that Toxocara eggs in public places are prevalent in regions with a high relative humidity, which are located at high longitudes and low latitudes (2). However, as sampling seasons and soil examination methods are different, it is not possible to reach a clear conclusion on the contamination of public areas in different localities of the country. A countrywide survey at one time period and a single method is suggested.

In the present study, the molecular examination was not performed to define the distribution pattern of Toxocara species in soil samples; however, there are two studies in Iran in which PCR followed by sequencing were used for this purpose. In Ahavz, 28.0% and 5.7% of the soil samples contained eggs of *T. cati* and *T. canis*, respectively (22). And, in Shiraz, 15.3% and 0.7% of soil samples were contaminated with T. cati and T. canis (19). There are controversies in the literature concerning *Toxocara* spp. in human patients. Some authors state that most cases of human toxocariasis have been associated with parasitism caused by T. canis (44), while some others believe that T. cati has been underestimated and as cats have more access to places where children go, T. cati should be considered responsible for the majority of larva migrans cases (7). In Iran, since the first report of larva migrans in 1976 (45), more than 25 confirmed cases of human toxocariasis including VLM, OLM, and NT have been documented as reviewed in a previous study (3). However, the species of Toxocara infecting patients in the country has not been investigated so far. In human populations, serologic tests are commonly used for diagnosis of toxocariasis; however, the problems of cross-reactivity in polyparasitism and indistinguishability between T.

canis and *T. cati* (or possibly *T. malayensis*) still exist (2). Species-specific diagnostic tools are required to support future epidemiological investigations. Moreover, surgeons should be encouraged to send the freshly removed worm body for molecular identification.

It is generally believed that only dogs and cats play role in the contamination of the environment. However, it has been shown that snails have roles in the epidemiology of toxocariasis (46). In a study in an open space in Buenos Aires, Argentina, 20% of *Rumina decollata* were infected with the third larval stage (L3) of *Toxocara cati* (46). A clinical trial on experimental infection of dogs/cats with infected snails will clarify the role of snails in toxocarosis of carnivores. Furthermore, the snail-to-snail transmission of nematodes larvae has been described as a novel route (47). It would be valuable to study whether or not "intermediesis" occurs for *Toxocara* species.

In most of the discussions, stray and pet cats and dogs are mentioned to be responsible for contamination of the environment but several species of wild canids (e.g. red fox, golden jackal, and wolf) and wild felids (e.g. lynx) are definitive hosts of *T. canis* and *T. cati* (48). It is suggested that foxes can be considered as the primary source of *Toxocara* spp. eggs in the environment because of the high prevalence of patent infections and a total absence of any anthelmintic treatment (49). Phylogenetic analyses of *Toxocara* spp. recovered from wild canids and felids are required to clarify the role of wild carnivores in contamination of the urban environments via their movements in borders of the cities and their potential to infect stray dogs and cats.

Conclusions

This study provides the first information about the contamination of public areas with *Toxocara* spp. in Hamedan province and calls for an increased general awareness of public health risk. Parents and pet-owners should get informed about the hazards of toxocariasis by medical and veterinary practitioners. Moreover, the media and science communicators should educate the society on prevention strategies to minimize the risks of zoonotic transmission. Further studies in the province

 Table 1. Published Studies on Contamination of Public Areas in Iran With Eggs of Toxocara spp. Until July 2020

Region	City	Source of Samples	Number of Examined Sites	Number of Soil Samples	Infection Rate ^a by Microscopy (%)	Infection Rate by PCR (%) and Identified Species	Year ^b	Reference
South	Shiraz	Public places and children's playgrounds	26	112	6.3	NI°	2002–2003	(17)
	Shiraz	Public parks	20	200	15.0	NI	2012	(18)
	Shiraz	Public parks and playgrounds	50	150	4	16: 15.3% <i>T. cati,</i> 0.7% <i>T. canis</i>	2015	(19)
	Jahrom	Public parks, elementary schools and kindergartens	ND^d	171		NI	2016	(20)
	Larestan	Children playgrounds	16	80	31.25 ^e	NI	2018	(21)
South-west	Ahvaz	Sidewalks, public parks, squares, and rubbish dumps	ND	210	30.5	33.8: 28% T. cati 5.7% T. canis	2011-2012	(22)
	Ahvaz	Public parks	ND	260	NI	21.9: 7.3% T. cati 14.6% T. canis	2019	(23)
	Abadan	Public parks	31	291	29.2	NI	2012	(24)
	Khorramshahr	Parks and green public areas	21	150	18	NI	2018-2019	(25)
North-west	Urmia	Public parks	26	102	7.8	NI	2003-2004	(26)
	Tabriz	Public parks	75	300	9.3	NI	2008-2009	(27)
	Ardabil	Sidewalks, public parks, squares, children's playgrounds and rubbish dumps	41	200	7	NI	2013-2014	(28)
	Tabriz	Public parks	60	540	34.4	NI	2013-2014	(29)
West	Khorramabad	Public parks	18	285	63.3	NI	2009	(15)
	Kermanshah	Public parks	7	150	18.0	NI	2014	(30)
	Kermanshah	Public parks, public streets, and squares	7	126	13.5	NI	2014	(31)
	Piranshahr	Public parks, soils around living space of guard, shepherd, and stray dogs	ND	150	8.0	NI	2012-2013	(32)
	Ilam	Public parks	10	40	40.0	NI	2018	(33)
		Public places, gardens, rubbish dumps	5	130	13.08	NI	2018-2019	(34)
	Hamedan	Public parks	20	120	11.7	NI	2019	This study
Center	Tehran	Parks, public places, and children's playgrounds	ND	150	38.0	NI	2008-2009	(35)
	Tehran	parks	120	600	10.0	NI	2008	(36)
	Isfahan	Public parks	28	140	28.6	NI	2014	(37)
	Arak	Public parks	15	60	26.6 ^d	NI	2015	(38)
	Karaj	Public parks	12	200	36.4	NI	2016	(39)
	Chaharmahal and Bakhtiari	Public places	74	180	5	NI	2017	(40)
	Tehran	Public parks	11	1132	11.39	NI	2017-2018	(41)
	Zanjan	Public parks	18	90	77.77 ^d	NI	2018	(42)
North-east	Mashhad	Dublic!	39	195	7.7	NII	2011 2012	(42)
	Khaf	Public parks	29	145	10.3	NI	2011-2012	(43)

^a Number of positive soil-samples/number of total soil samples; ^b Year of study; ^c NI: Not investigated, ^d ND: Not defined, ^e Prevalence value was calculated based on the total sampled sites.

especially with the aid of molecular-based studies for the identification of parasite species are suggested.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Ethical Approval

Not applicable.

Authors' Contributions

Conceptualization: AS, MZi; methodology: AS, ST, SN, SF, MZe; original draft preparation: AS; review and editing: MZi; funding acquisition: AS. All authors read and approved the final manuscript.

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