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Araştırma Makalesi ***Research Article***

Contamination of Soil Samples of Public Parks with *Toxocara* spp. Eggs in Kermanshah, Iran

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Abstract

Toxocariasis is a zoonotic helminth infection, occurring in humans by the accidental ingestion of embryonated eggs of *Toxocara canis* and less frequently *Toxocara cati*. The present study was conducted to determine the existence of *Toxocara* spp. eggs by using the sucrose flotation method. A total of 150 soil samples were collected randomly from 7 public parks in Kermanshah city between September and December 2014 for investigating the presence of infective stages of parasites and to determine the prevalence of helminth eggs. Of the 150 soil samples examined, 27 (18%) were infected with eggs of *Toxocara* spp. eggs. The present investigation showed that humans (especially children) from urban areas are at risk of acquiring helminth infection from contaminated soil. Since this host species is capable of transmitting zoonotic agents to both animals and humans, animal populations, including stray dogs and cats, have to be controlled to minimize the distribution of parasites.

Introduction

A large group of parasites occurring in the soil is called soil-transmitted parasites (Mandarino-Pereira et al., 2010). Stray dogs and cats are regarded as potential reservoirs of various parasites, e.g. gastrointestinal parasites, which can cause some infections in humans. They have also attracted increasing attention as the major sources of several frequent zoonoses, e.g. *Toxocara* spp., *Ancylostoma* spp. and *Cryptosporidium parvum* infections in urban areas (Acha and Szyfres, 2003; Mandarino-Pereira et al., 2010; Robertson and Thompson, 2002).

Toxocariasis is a zoonotic disease acquired through the accidental ingestion of the embryonated eggs of *Toxocara canis* (*T. canis*) and *Toxocara cati* (*T. cati*) existing in the feces of infected dogs and cats, respectively (Gurel et al., 2005). The larvae of the helminths can enter the human body from the soil or contaminated hands and also eating raw vegetables and undercooked giblets containing embryonated eggs (Magnaval et al., 2001). Based on the tissue attacked by the parasite, varying levels of harm and symptoms will manifest (Inan et al., 2006). *T. canis* eggs have been found to be highly resistant to unfavorable temperatures and the presence of chemical agents (Glickman and Shofer, 1987). Meanwhile, under optimal environmental conditions, embryonation of an egg to an infective stage (containing a second-stage larvae) will require about two to seven weeks (Lylod, 1998). As embryonated eggs are swallowed, they hatch in the intestine and the larvae move towards other organs and tissues. Although their destination is generally the liver and the lungs, they may locate in the kidney, heart, retina, and central nervous system. The migration of larvae to the viscera leads to a condition called visceral larva migrans (VLM). Serious ocular damage, known as ocular larva damage (OLM), can also be caused when the larvae to the retina (Dubna et al., 2007; Gillespie, 1993; Glickman and Schantz, 1981; Tavassoli et al., 2008). The infection is more common in children since they may play with soil, ingest dirty foods, lack of proper hygiene and are in frequent contact with dogs (Gurel, 2005).

In order for *Toxocara* spp. eggs to become infective, they need to be incubated in the soil for at least two weeks (Dubin et al., 1975; Paul et al., 1988). When remained in the soil, the embryonated eggs will be viable for long periods (Glickman and Schantz, 1981).

Numerous cases of child toxocariasis manifesting as endomyocarditis, generalized lymphadenopathy, endophthalmitis, asthma, hepatosplenomegaly and meningoencephalitis have been documented from the world over the past 10 years (Chan et al., 2001; De Cock et al., 1998; Kincekova et al., 1999; Szczepanski et al. 1996; Vidal et al., 2003). A study on healthy children in Zanjan (Northwest of Iran) revealed seropositivity for toxocariasis in 1.6% of urban and 4.4% of rural children (Nourian et al., 2008). Considering the insufficient studies on soil contaminated with *Toxocara* spp. eggs in public parks of Iran (Khazan et al., 2012; Saraei et al., 2012; Tavassoli et al., 2008; Zibaei et al., 2010), the present study aimed to determine the existence of *Toxocara* spp. eggs in soil samples from public parks in Kermanshah (Western Iran).

Materials and Methods

Study Area

Kermanshah province of Iran, covering an area of 24,990 square kilometers, is located in the west of the country. It is bordered by Iraq to the west and located beside two Iraq city of Soleimaniye and Diyali. Kermanshah province is located between latitude 34°18′ N and longitude 47°4′ E with an altitude of 1,350 m above sea level. Kermanshah has a moderate and mountain climate and the annual rainfall is 500 mm. The average temperature in the hottest months is above 22°C.

Samples

A total of 500 grams of soil sample from an area of 20 cm^2 in width with 10 cm in depth was collected from 7 public parks in Kermanshah from September to December 2014 (Figure 1). Twenty to twenty five samples were collected randomly from 3-4 various sites of each park. The soil samples from the same park were thoroughly mixed and stored in sealed and labeled polyethylene bags and taken to the laboratory. The recovery of *Toxocara* spp. eggs from the soil was performed using a modified flotation method from O'Lorcain (1994) with a little modification. To recover parasites, the soil samples were examined by Dunsmore et al. modified technique (Dunsmore et al., 1984) and by adaptation of Rugai's et al. method (Carvalho et al., 2005). Soil samples were dried for 2-3 days at room temperature and sifted through a 100 µm mesh sieve. Fifteen grams of sieved soil were transferred into a 50 ml centrifuge tube. Fifty milliliters of 0.05 % Tween 20 solution was added and vortexed vigorously for 1 minute and centrifuged at 1500 rpm for 3 minutes. The supernatant was discarded and 30 ml of flotation solution was added, mixed and 15 ml of the suspension was transferred into a 15ml centrifuge tube and centrifuged at 4000 rpm for 5 minutes. Later, a cover slip was left on the top of each tube for 15 min and then microscopic examination was performed for detection of *Toxocara* eggs at 100 X magnification. Pitting in *T. canis* is coarser than that in *T. cati.* Light microscopic observation yielded results.

Statistical Analysis

Descriptive statistics were used for data analysis.

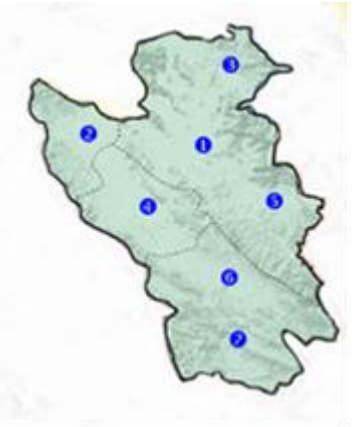

Figure 1. Map show the sampling area of 7 public parks in Kermanshah, Iran.

Results

A total of 150 soil samples were examined and *Toxocara* spp. (Figure 2) eggs were identified in samples by sucrose flotation method technique. Out of 150 samples examined, 27 were found positive for *Toxocara* spp. eggs with a prevalence of 18%. Overall, regarding the *Toxocara* spp. eggs in soil samples, mean egg number of positive samples was 12 eggs per sample (Table 1). The light microscopic investigations showed the presence of undeveloped eggs. All samples were negative for protozoa, *Strongyloides* spp., *Taenia* spp. eggs as well as hookworm and rhabditoid larvae.

Discussion

Following the recent increase in the number of cats and dogs in Iran, these animals are frequently seen in public parks of various cities of the country. As a result, the frequency of helminth eggs, which can substantially contribute to the incidence of zoonotic diseases, soil samples has elevated (Magnaval et al., 2001). Since *Toxocara* spp. eggs require an incubation period to become infectious, contact with contaminated soil is more risky than direct contact with infected cats or dogs. This is because eggs need a period to be incubated in soil to be infective. Meanwhile, the high prevalence of pica in children multiplies their chance of developing infections (Gurel et al., 2005).

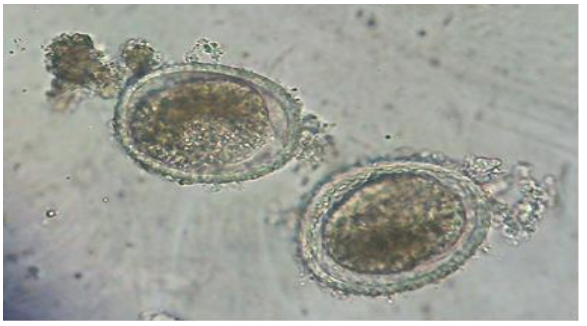

Figure 2. *Toxocara* spp. eggs from soil sample

Table 1. Mean numbers of eggs per sample according to the each public parks

Public Park No.	Number of Samples	Mean Numbers of Eggs/Sample
1	30	12.43±1.80
2	20	13.00±1.70
3	20	11.28±1.90
4	20	11.90±1.79
5	20	14.00±1.82
6	20	12.00±1.77
7	20	10.28±1.69
Total	150	12.12

We found *Toxocara spp.* eggs in 18% of the soil samples. Previous researches on soil samples from public parks in eastern Spain, Aydin of Turkey and Costa Rica have indicated similar findings (Gurel et al., 2005; Paquet-Durand et al., 2007; Ruiz et al., 2001). The mentioned rates of contamination with *Toxocara* spp. eggs in Kermanshah were lower than the rates reported from Frankfurt, Germany (87.1%), Thessaloniki, Greece (97.5%) and Sao Paulo, Brazil (60%) (Duvell et al 1984; Himons et al 1992; Santarem et al 1993). In Iran, however, even lower rates have been observed in Qazvin (5.8%) and Tehran (10%) (Khazan et al., 2012; Saraei et al., 2012). Such higher frequencies in other countries can be attributed to the greater number of pet dogs in their parks. In fact, due to cultural and religious reasons, owning and walking a dog are very rare in most cities of Iran including Kermanshah. Meanwhile, the higher frequency of parasite eggs in Kermanshah, compared to Qazvin and Tehran, can be justified not only by the large numbers of stray dogs and cats in Kermanshah, but also the greater prevalence of *Toxocara* spp. in the animals of the city.

In addition to the number of stray dogs and cats and cultural aspects, differences in testing methods, geographical parameters, sample collection periods and soil humidity might have also been responsible for the observed inconsistencies (Khazan et al., 2012; Nunes et al., 1994; Storey and Phillips, 1985).

While VLM can be caused by various organisms such as *Ascaris lumbricoides*, *Ascaris suum, T. cati* and *Capillaria hepatica*, *T. canis* is identified as the primary and most prevalent cause of the disease (Inan et al., 2006). However, in this study, eggs belonging to nematodes were not found. Only *Toxocara spp.* eggs were encountered in the current study. Likewise, the samples collected by Stojcevic et al. (2010) were found negative for both *Strongyloides* spp. and *Taenia* spp. eggs and larvae. In contrast, Tavalla et al. (2012) reported soil samples from public places in Tehran, Iran to be positive for the eggs of soil-transmitted helminth and protozoa. Such a disparity between the results of various studies can stem from differences in geographical locations of the study areas, composition, type, and humidity of soil, testing methods and of course, the number of stray animals, especially dogs (Khazan et al., 2012; Nunes et al., 1994; Storey and Phillips, 1985).

Conclusion

Although the results of the present study represent relatively low contamination with eggs of *Toxocara* spp. in the parks of Kermanshah, , it should be kept in mind that children always take a risk of visceral larva migrans while playing in contaminated playgrounds. Educational programs are needed to be initiated to prevent and control VLM infections in both rural and urban people. Clinicians also should consider the clinical features of VLM.

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