

Full Length Research Paper

Prevalence of parasites in soil samples in Tehran public places

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The aim of this study was to determine the prevalence of all parasitic forms (eggs, larvae, cysts and oocyst) by using two flotation methods in soil of public places and children's playgrounds in Tehran City, Capital of Iran. During 2008 to 2009, 150 soil samples were collected from various sites by simple random selection. To recover parasites, the soil samples were examined by sodium nitrate flotation, sucrose flotation method. The McNemar test and Kappa Index were used to analyse the statistical significance of the results. The prevalence of soil parasites was as follows: *Toxocara* spp. eggs in sodium nitrate flotation (38.7%) and in sucrose flotation method (33%), *Isospora* spp. in sodium nitrate flotation (10.7%) and in sucrose flotation method (18.7%), nematode larvae in sodium nitrate flotation (40.7%) and in sucrose flotation method (24%), *Eimeria* spp. in sodium nitrate flotation (8.7%) and in sucrose flotation method (24.7%), Coccidian oocyst and *Sarcocystis* spp. in sodium nitrate flotation (27%) and in sucrose flotation method (42%), *Dicrocoelium dendriticum* in sodium nitrate flotation (2.7%) and in sucrose flotation method (2%), Geohelminths in sodium nitrate flotation (6.7%) and in sucrose flotation method (3.4%). Furthermore, following sucrose flotation method performance, modified Ziehl-Neelsen staining technique was done and oocysts of *Cryptosporidium* spp. was detected in 15 (10%) of soil samples. According to McNemar test, the sodium nitrate flotation and sucrose flotation method statistically were differed to parasites detection. Results of our findings provide evidence that soil may play an important role in transmission of zoonotic parasite diseases to human. In addition, control of high population of animals such as stray dogs and cats is necessary to reduce the distribution of parasites.

Key words: Prevalence, parasites, flotation method, Tehran.

INTRODUCTION

Soil transmitted parasites are the large group of parasites that live in the soil during their development (Mandarino-Pereira et al., 2010). Contamination of soil with parasite eggs, infective larvae, cysts, and oocysts constitutes a most important risk factor for zoonotic parasite infection. Zoonotic parasites (that is, *Toxocara* spp.) and geohelminths (that is, *Ascaris lumbricoides*, *Trichuris*

trichiura and *hook worms*) are the main parasites that could be transmitted by soil (Waenlor and Wiwanitkit, 2007). According to prior reports, geohelminths were the second causes of mortality in children under six years of age in Africa (Ogbe et al., 2002).

Toxocariasis is a zoonotic disease caused by the larvae stage of *Toxocara cati* and *Toxocara canis*. Visceral larva migrant (VLM) and ocular larva migrant (OLM) are serious infections that are caused by these parasites. The presence of stray cats and dogs are the main sources of toxocariasis agent in the urban regions.

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Toxocara eggs require a period of four to six weeks incubation in soil to become infective (Paul et al., 1988; Dubin et al., 1975).

Many factors such as sample collection periods, methods of parasites recovery, number and volume of samples, humidity or desiccation of soil, are influencing the results of soil examination (Nunes et al., 1994; Storey and Phillips, 1985).

However, many studies carried out on frequency of parasites in soil samples in several parts of the world (Mandarino-Pereira et al., 2010; Rai et al., 2000; Uga et al., 1996). But, there is a few epidemiological data on prevalence of parasites in the soil samples of various parts of Iran. Zibaei et al. (2010) and Motazedian et al. (2006) studies carry out in Khorram Abad and Shiraz cities and they focused mainly on *Toxocara* spp. in soil.

The aim of this study was to determine the prevalence of all parasitic forms (eggs, larvae, cysts and oocyst) by using two flotation methods in soil samples of public places and children's playgrounds in Tehran.

MATERIALS AND METHODS

Sampling

During 2008 to 2009, 150 soil samples were collected from various sites in Tehran by simple random selection. At first, the town was geographically divided into five regions: north, south, east, west and center. Thirty samples were collected from each region. The study was focused on parks, public places and children's playgrounds. In each collection approximately 50 g was collected from 3 cm ground depth. As some samples were moist, thus all samples were air-dry at room temperature for approximately 24 h on tray.

Saturated sodium nitrate flotation

Isolation of eggs, oocysts and other parasitic forms was carried out for each sample by sodium nitrate flotation as described previously (Mizgajska-Wiktor, 2005) with some modifications. Briefly, the dried soil sample was mixed and sifted to remove solid objects. Then 20 g weighted sample was put into a 250 ml broad smooth opening Erlenmeyer's flask. Fifty milliliter of 5% sodium hydroxide (NaOH) (Merck, Germany) was poured into the sample and left for 1 h to separate eggs from the soil. Then, the sample was shaken for 20 min. Whole content of the flask was energetically poured into a 50 ml falcon tube. The sample was centrifuged for 3 min with 1500 rotations per minute (rpm) in order to settle the eggs and oocysts on the bottom. The supernatant was discarded and the sediment was washed three times with distilled water. After final washing the sediment was resuspended in saturated sodium nitrate (NaNO_3) (Merck, Germany) with specific gravity 1.30 and centrifuged again (1500 rpm, 3 min). The tube was transferred into the stand, and the flotation fluid was added to the tube with a pipette until the fluid raised up to the brim of tube. Then on the surface of the fluid a 24 × 24 mm cover slip was placed and left for 30 min. During this time, parasitic eggs and oocysts stick to the glass. The cover slip with the hanging drop on the underside is placed on the slide and the specimen was prepared for microscopic observation.

Sucrose flotation method

Isolation of eggs, oocysts and other parasitic forms was performed

for each sample by sucrose flotation method as described previously (Rai et al., 2000) with some modifications. Briefly, 4 g soil sample was dissolved in 50 ml distilled water and centrifuged in 1000 rpm for 5 min. Then the supernatant was discarded and sediment was resuspended in 30 ml distilled water in a falcon tube and layered over with 15 ml sucrose (Merck, Germany) solutions with specific gravity of 1.40. After centrifugation at 800 × g for 5 min, the interface and the upper layer of liquid was transferred to a new tube, and centrifuged at 1000 rpm for 5 min. The sediment was resuspended in 50 ml distilled water and centrifuged at 5000 rpm for 5 min. The sediment was transferred to 1.5 ml microtube and the trace of remaining sucrose was removed by two times washing with distilled water. The final sediment was used for direct microscopic examination.

Modified Ziehl-Neelsen staining

Cryptosporidium oocysts were identified by the sucrose flotation method followed by the modified Ziehl-Neelsen staining technique (John and Petri, 2006).

Data analysis

The McNemar chi-square test and Kappa Index were used for association and measuring the agreement between the results of each flotation method applied to recover parasites, respectively (K < 0.2 poor agreement, K 0.2 to 0.4 fair agreement, K 0.41 to 0.6 moderate agreement, K 0.61 to 0.8 good agreement, K 0.81 to 1.0 very good agreement) (Altman, 1992).

RESULTS

Of the total 150 soil samples collected from five regions of Tehran, 119 (79.3%) were found to be positive for parasites (Figure 1). The prevalence of parasites in soil samples are summarized in Table 1. *Toxocara* spp. egg was more prevalent than others parasite in soil samples of Tehran. *Cryptosporidium* spp. oocysts was detected in 15 (10%) of soil samples. In Table 1, we compared two methods for separation of parasites and prevalence of them, but we identified *Cryptosporidium* by sucrose method only. The McNemar test indicated that, there were statistically significant differences between results of two flotation methods in recovering *Toxocara* spp. eggs ($p = 0.006$), *Isospora* spp. oocyst ($p = 0.000$), Coccidian oocysts ($p = 0.000$), *Eimeria* spp. oocysts ($p = 0.000$) and nematode larvae ($p = 0.000$) in soil samples (Table 1). Kappa coefficient indicated moderate or good agreement between two methods to recover the most of parasites.

DISCUSSION

This work was the first epidemiological study on prevalence of on all parasitic forms by using two flotation methods in soil samples of public places and children's playgrounds in Tehran.

Previous studies showed 6.3% and 22.2% prevalence of *Toxocara* eggs in soil samples in other cities of Iran

Table 1. Comparison of parasites prevalence detected by two flotation techniques in 150 soil samples in Tehran public places.

Parasite	Technique		Statistical analysis	
	Sodium nitrate flotation	Sucrose flotation	McNemar χ^2	Kappa (STD error)
	N (%)	N (%)	(P value)	(K)
Helminths				
<i>Toxocara</i> spp. eggs	58 (38.7%)	48 (33.0%)	0.006	0.826 (0.048)
Geohelminth eggs	10 (6.7%)	5 (3.4%)	0.063	0.651 (0.144)
<i>Dicrocoelium</i> eggs	4 (2.7%)	3 (2.0%)	1.000	0.854 (0.144)
Nematode Larvae	61 (40.7%)	36 (24.0%)	0.000	0.601 (0.064)
Protozoa				
<i>Eimeria</i> spp. oocyst	13 (8.7%)	37 (24.7%)	0.000	0.449 (.086)
<i>Isospora</i> spp. oocyst	16 (10.7%)	28 (18.7%)	0.000	0.684 (.083)
Coccidian oocyst and <i>Sarcocystis</i> spp.	27 (18.0%)	42 (28.0%)	0.000	0.722 (.065)

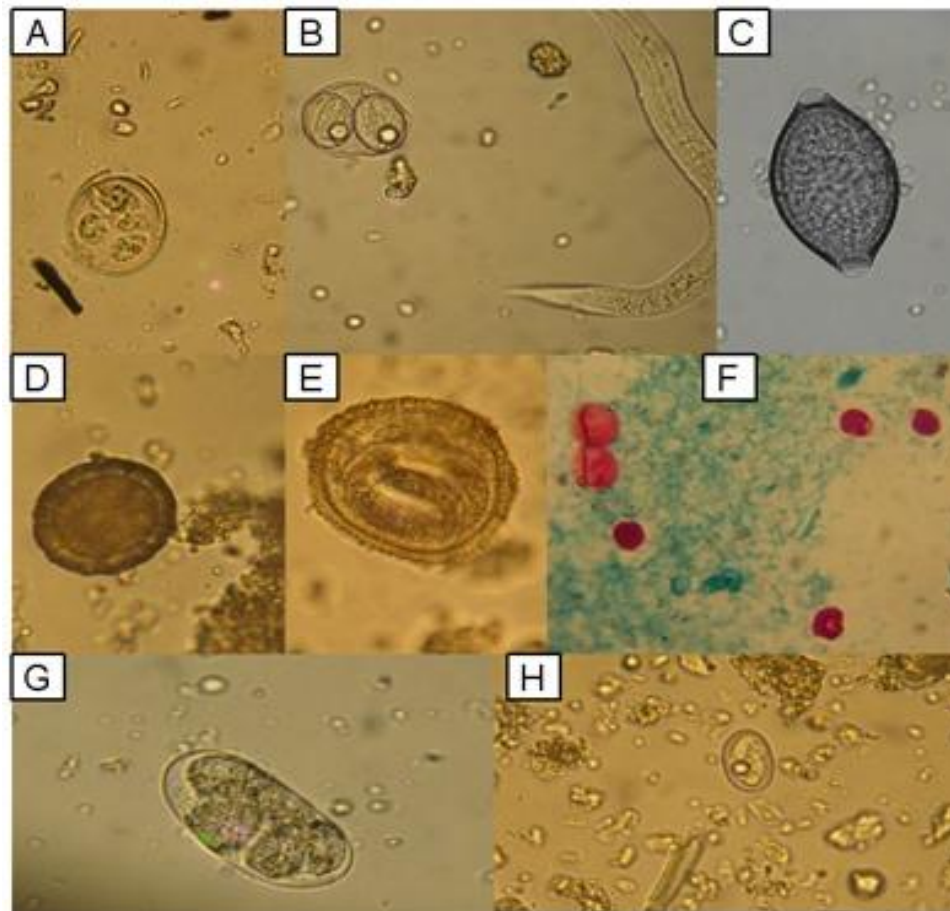


Figure 1. Parasites in soil samples in Tehran public places. A, *Eimeria* oocyst; B, Coccidian oocyst and nematode larvae; C, *Trichocephalus* egg; D, *Ascaris* egg; E, *Toxocara* spp. egg; F, *Isospora* and *Cryptosporidium* oocysts; G, hook worm egg; H, *Sarcocystis* spp.

(Motazedian et al., 2006) in Shiraz and Khorram Abad (Zibaei et al., 2010). The prevalence of *Toxocara* in our

study was higher than earlier studies. This fact might be due to different climate conditions or diagnostic methods

that was used in our experiments. Eggs of *Toxocara* spp. are resistant to environmental conditions and can remain transmittable for several years in favorable condition. Tiyo et al. (2008) also reported a high rate of *Toxocara* egg contamination in soil samples from public squares in southern Brazil.

Our study shows that *Toxocara* spp. were the most common parasites in soils of public places in Tehran, high prevalence of VLM/OLM caused by this parasite is expected, especially in children. However, other studies have shown that the seroprevalence of toxocariasis in this group is not remarkable (unpublished data). It may be due to lack of appropriate diagnostic methods or limited studies.

Eggs of geohelminths including *Ascaris* spp., *Trichuris* spp. and hook worms need a period of time, outside the host body to develop and attain infective stage. Presence of these parasites in the environment can be a public health indicator (Saathoff et al., 2002). Low prevalence of these parasites in our study (Table 1) indicates relative good environment hygiene. Furthermore, the use of human feces as fertilizer which was avoided could be a factor of low frequency of finding these parasites in parks and other parts of urban areas.

Coccidian parasites, including *Isospora* spp., *Eimeria* spp. and others, have animal origins and are capable of contaminating the environment through feces of dogs, cats and birds.

Contaminations of soil with Coccidian oocysts that may be belonging to *Hammondia* sp., *Neospora* sp. and *Toxoplasma gondii* are epidemiologically important as they may contribute in maintaining parasite cycle in nature and providing a source of infection for human or other animals.

Although, research on prevalence of *Cryptosporidium* oocysts in soil samples was rarely found, it was confirmed from different soil types (Mawdsley et al., 1996). *Cryptosporidium* oocysts are resistance to environmental conditions, for example, oocysts could tolerate to low temperature, even -10°C and still be infective to human and animals (Fayer, and Leek, 1984). In our study *Cryptosporidium* oocysts isolated from soil samples which exhibited relatively high prevalence (10%) should indicate attention to public health sector.

Conclusion

In conclusion, results of our findings on soil contamination by parasites in public places and other urban area indicate that human health risk and may play an important role in transmission of these zoonotic parasite diseases to human. In addition, control of population of animals such as stray dogs and cats is necessary to reduce the distribution of parasites.

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