# Prevalence of parasites in soil samples in Tehran public places

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**Abstract:** The aim of this study was to determine the prevalence of all parasitic forms (eggs, cysts, oocyst, and larvae) by using two flotation methods in soil of public places and children's playgrounds in Tehran City, Capital of Iran. During 2008-2009, 150 soil samples collected from various sites in Tehran by simple random selection. To recover parasites, the soil samples were examined by sodium nitrate flotation, sucrose flotation method and modified Ziehl-Neelsen staining technique. The McNemar test and Kappa Index were used to analysis 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 *Sarcosystis* 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.
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## 1. Introduction

Soil transmitted parasites are the large group of parasites that live in the soil during their development to find proper host (Mandarino-Pereira et al., 2010). Contamination of soil with parasite eggs, cysts, and oocysts as well as the penetration of skin by infective larvae constitutes a most important risk factor for zoonotic parasite infection. Zoonotic parasites (i.e Toxocara spp.) and geohelminths (i.e Ascaris lumbercoides, Trichuris trichiura and Hook worms) are main parasites that could be transmitted by soil (Waenlor & Wiwanitkit, 2007). According to prior reports, geohelminths are 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. The presence of stray cats and dogs are the main sources of toxocariasis agent in the urban regions. Visceral larva migrant (VLM) and ocular larva migrant (OLM) are serious infections that caused by Toxocara spp. eggs and required a period of 4-6 weeks incubation in soil to become infective (Paul et al., 1988; Dubin et al., 1975).

Many factors such as the season of sampling, 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 & 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. Most of the studies have focused mainly on prevalence of Toxocara spp. eggs in public places (Zibaei & Sadjjadi, 2010; Motazedian et al., 2006; Zibaei et al., 2010).

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

## 2. Material and Methods

### Sampling

During 2008-2009, 150 soil samples collected from various sites in Tehran by simple random selection. At first, the town geographically was divided to five regions: the North, South, East, West and center. The study was focused on parks, public places and children's playgrounds. Thirty samples collected from each region, weight of each sample was approximately 50g and site of collection was 3 cm ground depth. As some samples were moist, all of them spilt out on tray and depend to soil humidity, remained approximately 24 hours in room temperature. Helminths eggs or larvae and protozoan oocysts were identified duo to morphological and measure characteristics.

### 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 a 20g portion was weighted and put into a 250 ml broad smooth opening Erlenmeyer's flask. To separate eggs from the particles of soil 50 ml of 5% sodium hydroxide (NaOH) (Merck, Germany) was poured into the sample and left for 1 hour. Then, the sample was shaken for 20 minutes. Whole content of the flask was energetically poured into a 50 ml falcon tube. To settle the eggs and oocysts on the bottom, the sample was centrifuged for 3 minutes with 1500 rotations per minute (rpm). The supernatant is discarded and sediment washed three times by distilled water. After washing the material the sediment was suspended in saturated sodium nitrate ($NaNO_3$) (Merck, Germany) with specific gravity 1.30 and centrifuged again (1500 rpm, 3 minutes). The eggs and other parasitic forms should set off on the surface. The tube is transferred into the stand, and the flotation fluid is added with a pipette. Then on the surface of the fluid a 24 × 24 mm cover slip was placed and left for 30 minutes. 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.

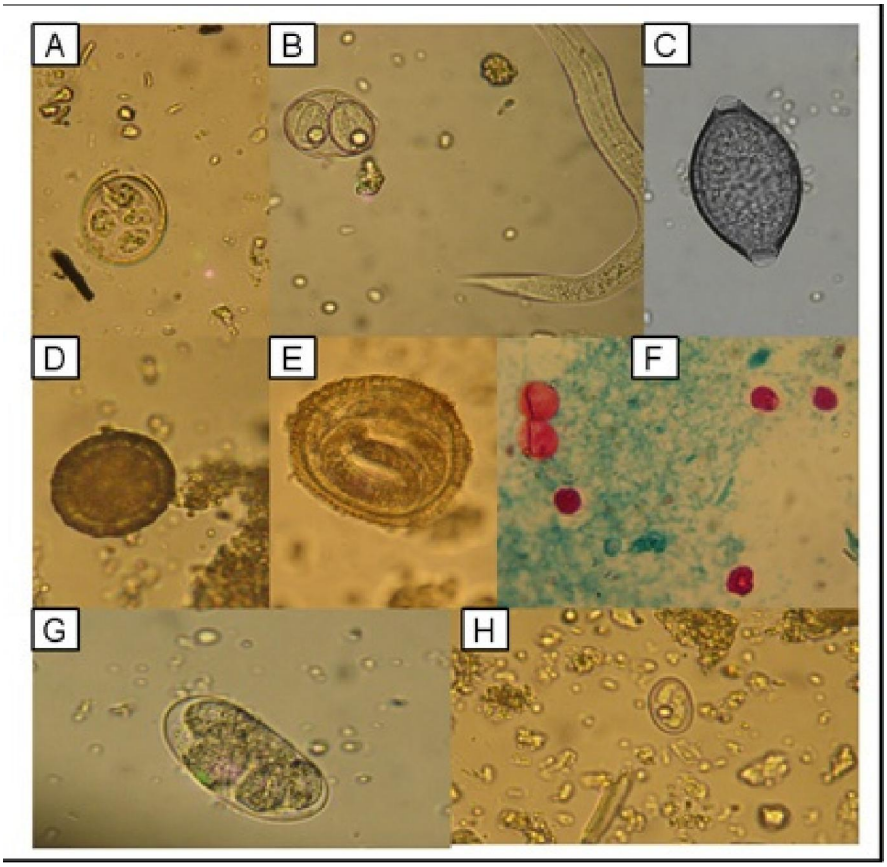


Fig 1: Prevalence of parasites in soil samples in Tehran public places.
A: *Eimeria* oocyst; B: Coccidian oocyst and nematode larvae; C: *Trichocephal* egg; D: *Ascaris* egg; E: *Toxocara* spp. egg; F: *Isospora* and *Cryptoporidium* oocysts; G: Hook worm egg; H: *Sarcocystis* spp.

**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 suspended in 30 ml distilled water and layered over 15 ml sucrose (Merck, Germany) solutions with specific gravity 1.40 in a falcon tube. 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 suspended 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 removed by two times washing with distilled water. The final sediment was used for direct microscopic examination to recover parasites.

**Modified Ziehl-Neelsen Staining**

*Cryptosporidium* oocysts were identified by the sucrose flotation method followed by the modified Ziehl-Neelsen staining technique (John & 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 0.2–0.4 fair agreement, K 0.41–0.6 moderate agreement, K 0.61–0.8 good agreement, K 0.81–1.0 very good agreement) (Altman, 1992).

**3. Results**

Of the total 150 soil samples collected from five regions of Tehran, 119 (79.3%) were found to be positive for parasites (Fig 1). The prevalence of parasites in soil samples are summarized in Table 1. According to this table, *Toxocara* spp. egg was more prevalent parasite than others in soil samples of Tehran. *Cryptosporidium* spp. oocysts was detected in 15 (10%) of soil samples. The McNemar test indicated that, there were statistically significant differences between results of two flotation methods to recover *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 (Table 1).

Table 1: Comparison between prevalence of parasites in soil samples by two flotation techniques in Tehran public places

| **Parasites** | **Techniques** | | **Statistical Analysis** | |
|---|---|---|---|---|
| | Sodium Nitrate Flotation | Sucrose Flotation | McNemar $\chi^2$ | Kappa (std Error) |
| | N (%) | N (%) | (*P* value) | (*K*) |
| Helminths | | | | |
| *Toxocara* spp. eggs | 58 (38.7%) | 48 (33.0%) | .006 | .826 (.048) |
| Geohelminth eggs | 10 (6.7%) | 5 (3.4%) | .063 | .651 (.144) |
| *Dicrocoelium* eggs | 4 (2.7%) | 3 (2.0%) | 1.000 | .854 (.144) |
| Nematode Larvae | 61 (40.7%) | 36 (24.0%) | .000 | .601 (.064) |
| Protozoa | | | | |
| *Eimeria* spp. oocyst | 13 (8.7%) | 37 (24.7%) | .000 | .449 (.086) |
| *Isospora* spp. oocyst | 16 (10.7%) | 28 (18.7%) | .000 | .684 (.083) |
| Coccidian oocyst & *Sarcosystis* spp. | 27 (18.0%) | 42 (28.0%) | .000 | .722 (.065) |

**4. Discussions**

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

Since our study showed that *Toxocara* spp. are the most common parasites in soils of public places in Tehran, we expected to have a high prevalence of this parasite, 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.

Previous studies that were carried on frequency of *Toxocara* eggs in soil samples at other cities of Iran, reported the prevalence of 6.3% and 22.2%

(Motazedian et al., 2006; Zibaei et al., 2010) in Shiraz and Khorram Abad, respectively. The prevalence of *Toxocara* eggs in our study was higher than earlier studies. This fact might be duo 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 location. Tiyo et al. also reported a high rate of *Toxocara* contamination in soil samples from public squares in southern Brazil (Tiyo et al., 2008).

Eggs of Geohelminths including *Ascaris* spp., *Trichuris* spp. and Hook worms need a period of time, outside the host body to develop their life cycle and become infective. 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 (Table1) indicates improvement of environment hygiene. Furthermore, not using of human feces as fertilizer can be a cause for low frequency of these parasites in parks and other parts of urban areas.

Coccidian parasites, including *Isospora* spp., *Eimeria* spp. and others, have animal origins and contaminate 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 to maintain a parasitic cycle in nature and provide a source of infection for human or other animals.

Although, there was not enough research about prevalence of *Cryptosporidium* oocysts in soil samples, but it was confirmed that oocysts are isolated from different soil types (Mawdsley et al., 1996). *Cryptosporidium* oocysts are resistance to environmental conditions, for example, oocysts could tolerate low temperature, even -10º C and still become infective for human and animals (Fayer, & Leek, 1984). As in our study *Cryptosporidium* oocysts isolated from soil samples have relatively high prevalence (10%), so indicate attention to public health.

In conclusion, results of our findings provide evidence that soil contamination by parasites in public places and other urban area can affect human health and 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.

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