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Parasitic contamination of surface and deep soil in different areas of Sari in north of Iran

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ABSTRACT

Objective: To study the parasitic contamination of soil in selected areas of Sari, north of Iran.**Methods:** A cross-sectional study was conducted to identify all available parasites in surface and deep soil. In this study 580 soil samples (278 deep soil and 302 topsoil samples) from 21 different locations were collected from pathways, parks, greenhouses, estates around the city, cemetery, main squares, farmlands, fenced gardens and seashores. Depending on the soil type, two samples were prepared, from surface and deep soil at the depth of 3 to 5 cm. After performing various stages of preparation, including cleaning and washing, smoothing and flotation, parasitic elements were examined microscopically and quantitative parasite counting was done using a McMaster slide.**Results:** The results showed that the highest rate of parasitic contamination was related to nematodes larvae (26.11%). Other contaminants such as *Entamoeba* and *Acanthamoeba* cysts, vacuolization *Blastocystis hominis* form, oocyte containing sporocysts, *Toxascaris* eggs, nematoda larvae, *Hymenolepis* eggs, *Ascaris* eggs, *Fasciola* eggs, hookworm eggs, *Toxocara* eggs, insects' larvae and other ciliated and flagellated organisms were also observed. The results of this study showed that the highest contamination was found in public garden (25.80%) both in surface (29.30%) and in deep soil (21.12%), while the lowest level of contamination was observed in seashore surface soil (4.90%).**Conclusions:** The results showed that soil can provide a potential medium for the spread of soil transmitted parasitic diseases in the environment; therefore, preventive programs are needed.

1. Introduction

Transmissible diseases between humans and animals have ever been one of main health problems in the country which drained human and financial resources and so often appeared as a serious crisis in the form of emerging and re-emerging diseases[1]. Only soil transmitted helminthes and schistosomes affected more than one billion people in all of the world[2]. According to World Health Organization report, 832 out of 1709 pathogens are transmitted from

animals to humans[3]. Soil provides a suitable medium for biological processes necessary for the growth of majority of zoonotic parasites. Furthermore, soil provides the parasites with appropriate biological conditions, hosts and shelter to ensure their continuous existence and development as well as geographic dispersion in an area[4].

Among the parasites, soil-transmitted helminthes are more common than other parasites. During the last 50 years, despite significant advances in the fields of sanitary and medicine, not only the absolute number of infected people has not diminished but also the number of infected people has increased particularly in the so called developing countries mainly due to continued population growth[5]. Studies have shown that parasitic infections transmitted via the soil such as *Toxocara* are still prevailing in various provinces in the country both in urban and rural areas[1]. The parasitic infections of the highest prevalence in the world are *Ascaris*, hook worms and whip worms, all of which are soil-transmitted helminthes[6].

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The use of humans excreta as fertilizer in agriculture, discharging untreated wastewater in the environment, lack of sanitary catchment in rural areas, open defecation around human dwellings, parks and farms as well as low public awareness are all behinds the widespread contamination of soil with worms egg and protozoan cysts, This subsequently causes cysts, eggs or infectious larvae to find their ways back to humans particularly to young children when playing in contaminated grounds[7]. Therefore, identification of soil contamination in areas of parasitic transmission risks to humans is an important step towards establishing appropriate control and prevention strategies.

2. Materials and methods

2.1. Sample collection

The studied areas are located in Sari, Mazandaran Province, Iran. (Figure 1). A total of 580 soil samples were collected from 21 areas. Sampling of the soil was undertaken to differentiate infected areas and to determine the prevalence of parasites in the soil.



Figure 1. The scope of the study area in north of Iran.

To collect the soil samples, 21 points of the city were randomly selected. To ensure an even distribution of selected areas in the whole city, surface and deep soil samples were taken from 3 crowded passages of various traffic levels, 3 parks and gardens, 2 greenhouses, 2 towns around the city's squares, 1 cemetery, 3 main squares, 1 agricultural land, 5 fenced gardens within governmental offices as well as seashores of the Caspian Sea near the city of Sari. Samples were collected so that 80 were from passageways (crowded areas near the trash bins), 50 were from the seashore, 45 were from greenhouses, 80 were from public parks and gardens (children's playing areas and fertilized soil with animal manure), 75 were from urban settlements (next to sewage system), 70 were from main squares (fertilized with animal manure), 70 were from agricultural lands (fertilized with human waste), 50 were from fenced gardens, and 60 were from a cemetery (graveyard and graves surrounding).

2.2. Procedure and method

Two soil samples (30 g each) per locality were prepared from

surface and deep soil at the depth of 3 to 5 cm. The samples were separately added to 10-L graduated bucket containing 2 L of water, vigorously shaken and left to settle for 15 min. After the rest time, resultant sediment was consecutively passed through 1 mL, 100- μ m and 37- μ m sieves. To avoid any possible loss of parasites during filtration, a bucket were placed under the sieves to collect the parasites. Then, the sieves were carefully washed under water and the sediments were left to settle in an Imhoff funnel for 1 h. In this stage the filtered through supernatants were discarded and the sediments were added to the funnel to minimize the errors. After a stationary period of 1 h, the supernatants were discarded and the sediments transferred into centrifuge tubes and centrifuged at 3000–4000 r/min for 5 to 7 min. The centrifuged supernatants were removed and the remaining was dissolved in sucrose solution and centrifuged at 1000 r/min for 1 min. Immediately after this stage, the supernatants were filtered through a 37- μ m sieve and washed carefully into a clean container to remove the soluble sucrose and prevent plasmolysis[8]. The samples were then transported to the laboratory for qualitative and quantitative investigation of parasites using a microscope and a McMaster slide, respectively[9].

3. Results

A total of 580 soil samples from 21 areas of Sari were analyzed of which 278 ones were from deep soil and 302 ones were from surface soil. The investigation revealed the presence of parasites such as *Blastocystis* (11.20%), *Hymenolepis* eggs (0.55%), *Acanthamoeba* cysts (0.93%) *Amoeba* cysts (2.05%), fertile *Ascaris* eggs (0.74%) and *Toxocara* eggs (3.73%), all of which are human pathogens (Table 1).

Table 1

The number and percentage of all kinds of parasites isolated from soil.

Types of parasites	Number (%)
Oocysts	12400 (23.13)
<i>Blastocystis</i>	6000 (11.20)
<i>Fasciola</i> eggs	200 (0.40)
<i>Hymenolepis</i> eggs	300 (0.55)
Ciliate	6000 (11.20)
Nematoda larva	14000 (26.11)
<i>Acanthamoeba</i> cysts	500 (0.93)
Insect larvae	4500 (8.39)
<i>Amoeba</i> cysts	1100 (2.05)
Flagellate	5000 (9.32)
<i>Toxascaris</i> Eggs	400 (0.74)
Infertile eggs of <i>Ascaris</i>	500 (0.93)
<i>Ascaris</i> eggs	400 (0.74)
Hookworm eggs	100 (0.18)
<i>Toxocara</i> eggs	2000 (3.73)
Moniezia	200 (0.37)
Total	53600 (100.00)

3.1. Passageways contamination

The highest rates of infection was found to be related to oocysts (47.61%) and the lowest related to *Hymenolepis* eggs (1.58%). In deep soil, maximum and minimum pollutions were due to Nematode larvae (43.47%) and *Acanthamoeba* (10.86%), respectively.

3.2. Parks and gardens contamination

The highest rates of infection amongst the whole samples, parks and gardens surface soils were mainly occupied by nematode larvae (33.70%) and to lesser degree by oocysts (4.49%). The deep soils were polluted by oocysts (61.22%) and to a lesser extent by *Ascaris* eggs (8.16%).

3.3. Greenhouses contamination

Moniezia (9.09%) was the contaminant of the lowest presence in deep soil of greenhouse, while *Blastocystis* (68.18%) was the main pollutant.

3.4. Seashore contamination

Sea surface was found to be contaminated mainly by oocysts (66.67%) and to lesser degree by larvae of nematodes (33.33%).

3.5. Settlements contaminations

Nematodes larvae (50%) and cilia (50%) have the same prevalence in the deep soil of settlements. However, the surface soil was mainly occupied by oocysts (71.42%) and to a lesser extent by *Ascaris* (4.76%).

3.6. Cemeteries contamination

In the cemetery surface soil, the highest and lowest levels of pollution belonged to larvae of nematodes (74.07) and *Ascaris* eggs (7.40) respectively. Deep soil, however, contained *Blastocystis* (66.67%) and oocysts (33.33%).

3.7. Main city square contamination

The surface soil of squares was mainly polluted with flagellates

(44.44%) and to lesser degree with ciliate (22.22%). The deep soil, however, contains *Blastocystis* (20.00%), nematodes larvae (40.00%) and oocysts (40.00%).

3.8. Farmland contamination

The maximum and lowest rates of pollution on the surface of farmland is related to *Blastocystis* (43.47%) and hookworm eggs (4.34%) respectively. No parasitic infection was found in deep soil samples obtained from farmlands.

3.9. Fenced gardens contamination

The highest rate of infection in deep soil of governmental gardens belonged to nematode larvae (57.14%) and lowest rate was due to insect larvae (14.28%). No pollutants was found on the surface soil samples of these gardens.

Some of separated parasites were in Figure 2. The total number and percentage of parasites in surface and deep soils in different areas were shown in Table 2. Based on the above table the highest and the lowest prevalence were related to nematode larvae and hookworm respectively.

4. Discussion

Various studies were done on parasitic infections transmitted to humans through contact with soil. In Sari soils taken from 21 localities, the most abundant parasite was nematodes larvae and the less observed parasite was *Fasciola* eggs. Parks and gardens have the most parasitically polluted soils while the governmental fenced gardens and seashores have the less polluted ones. The results of this study revealed high risk of parasite transmission for urban people who frequently went green spaces and therefore ringing an alarm for whole society. Studies undertaken in Latin America, particularly

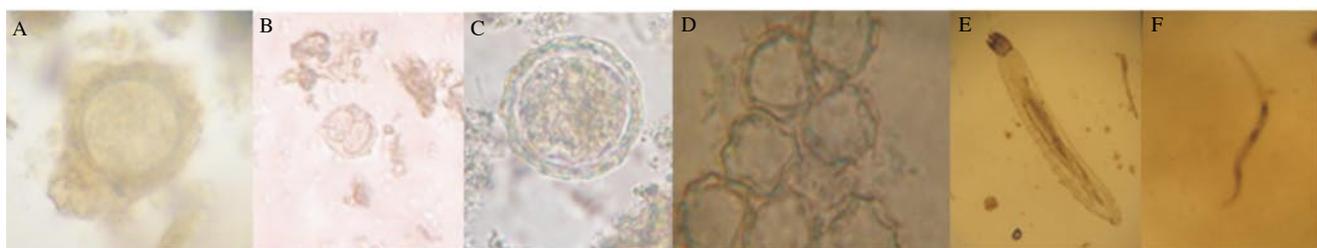


Figure 2. Separated parasites from the soil are: A: *Ascaris* egg; B: Oocyst; C: *Toxocara* egg; D: *Acanthamoeba* cysts; E: Insect larvae; and F: Nematode larvae.

Table 2

Variety, total number and percentage of parasites in surface and deep soils in different areas.

Area	Total parasites [n (%)]	Variety of parasites	Parasites in surface soil [n (%)]	Parasites in deep soil [n (%)]
Passageways	10900 (20.33)	9	6300 (20.70)	4600 (19.82)
Gardens	13800 (25.80)	9	8900 (29.30)	4900 (21.12)
Greenhouses	2200 (4.10)	3	–	2200 (9.49)
Seashores	1500 (2.79)	2	1500 (4.90)	–
Settlements around cities	8200 (15.29)	5	4200 (13.80)	4000 (17.24)
Cemetery	4200 (7.83)	5	2700 (8.90)	1500 (6.47)
Main city square	7000 (13.05)	5	4500 (14.90)	2500 (10.78)
Farmland	2300 (4.29)	5	2300 (7.50)	–
Government offices gardens	3500 (6.52)	3	–	3500 (15.08)
Total	53600 (100.00)		30400 (100.00)	23200 (100.00)

in Peru, Brazil, and in European countries as well as in some Asian and African regions, showed the soil polluting organisms are mainly worm's eggs, particularly *Ascaris* and *Toxocara*[10-12]. Children are the most vulnerable group to the parasitic infection due to their increased contact with soil when playing in open areas or because of pica resulting from lack of vitamins and minerals that occurs in some children. Previous studies undertaken in the city of Sari showed that infection by soil transmitted parasites such as *Ascaris* and *Toxocara* was continuing. In this study about 3.73% of soil samples were contaminated with *Toxocara* eggs which in less than the rates reported by other authors[13]. A study carried out in Tehran showed that 79.3% of the soil samples had parasitic contamination in which *Toxocara* was the main contaminant comprising 33.0% to 38.7% as revealed by sodium nitrate and sucrose flotation methods, respectively[14]. According to a study conducted in Shiraz (south of Iran) in 2013 on soil samples obtained from 20 public parks, *Toxocara* eggs was observed in 15% of the samples[10]. A study conducted in Hamadan (center of Iran) in 1997, on soils collected from 19 public parks revealed that 60.3% of the samples were infected by soil transmitted worms eggs of which 57.8% were *Ascaris* eggs and 36.8% were *Toxocara* eggs. Also, another study undertaken in Tabriz (north west of Iran) in 2012, to examine 300 soil samples taken from 75 public parks, indicated that *Toxocara* eggs occur in about 9.3% of the samples. The low occurrence of *Toxocara* in Tabriz study as well as in our study as compared to the aforementioned studies may be attributed to higher number of soil samples collected in the formers from diverse localities both from human and animal places which may dilute the overall presence of the parasite in the studied soils[15].

Despite the rising standard of public health and socio-economic conditions in Sari, building a limited number of waste water catchments in the city and people religious behavior of avoiding contact with dogs and cats, the parasitic infections are still prevailing. The most significant of these parasites are *Toxocara* eggs, *Toxascaris* egg, *Ascaris* eggs, hookworm eggs, and *Cryptosporidium* which cause zoonotic diseases. It is, therefore, necessary to reduce the risk of soil transmitted parasitic infections by observing good personal hygiene habits such as washing hands and washing vegetables as well as using fertilizers and compost in farmlands instead of other polluting waste materials. However, environmental sanitation remains the main control strategy in this concern. In rural regions where modern sewage systems are not available, digging private sewer pits may be encouraged. Use of sewage and waste material to fertilize lands in which represents an economic and health problem should be stopped unless they are biologically or chemically disinfected. Also, removing stray animals such as homeless cats and dogs in the city is an important step to reduce infection. Although this study proved the existence of relatively low contamination levels with *Toxocara* eggs and other parasites in Sari, further studies are required to trace sources of contamination and the ways to adopt effective prevention and control programs given the ongoing prevalence and fast spreading of soil-borne parasitic diseases

Conflict of interest statement

We declare that we have no conflict of interest.

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