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Cystoisospora spp., Contaminated Soil: The Potential Risk of Infection for the Public in the West of Iran

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Abstract

Cystoisospora spp., formerly known as *Isospora* spp., are coccidian parasitic protozoan with several species infecting a wide variety of organisms such as humans, felines and canines. This study was designed to evaluate the prevalence of *Cystoisospora* spp. oocysts in the soil collected from Kermanshah city, the west of Iran. One hundred and ninety-two soil samples were collected from six regions of Kermanshah city, including two regions in the city center as well as one region in each of the east, south, north and west regions. Regarding that this study was first of its kind in this region, the sampling method was according to judgmental sampling. Overall, from each region, 32 soil samples were collected, 16 of which were obtained from public parks and 16 other ones from primary schools. The *Cystoisospora* spp. oocysts were isolated from soil samples using the flotation method, then stained temporarily and permanently with Lugol's solution and modified Ziehl-Neelsen methods, respectively, and examined under a light microscope. Out of 192 soil samples, 15 (7.8 %) cases were infected with *Cystoisospora* spp. oocysts. In the public parks, the highest prevalence of oocysts was observed in the center 1 and the south regions, both with 12.5% prevalence. Moreover, in the primary school regions, the highest prevalence was observed in the center 2 (18.75 %) region followed by the east and west (both 12.5 %) regions. The findings of the present study revealed the potential of *Cystoisospora* spp. transmission in the west of Iran, and that the contamination of soil in primary schools was higher than public parks.

Keywords: Soil, Kermanshah City, Iran, *Cystoisospora* spp.

1. Introduction

Soil-transmitted parasites are a large group of parasites that live in the soil during their development (1). Contamination of soil with parasite eggs, infective larvae, cysts and oocysts constitutes the most important risk factor for zoonotic parasitic infection. Zoonotic parasites are the main parasites that could be transmitted by soil (2). *Cystoisospora* spp., formerly known as *Isospora* spp., are coccidian parasitic protozoan with several species infecting a wide variety of organisms such as humans, felines and canines (3). *Cystoisospora* spp. are ingested with contaminated food or water, and their life cycle requires a stage outside the host. After that *Cystoisospora* oocysts are ingested, they release sporozoites (possibly in response to bile in the small intestine), which invade the enterocytes of the proximal small intestine. Here, they become trophozoites, and asexual multiplication (schizogony) produces merozoites, which invade previously uninfected cells (4). Shortly thereafter, a sexual multiplication cycle (sporogony) begins, generating oocysts that may pass into the environment. Outside the host, oocysts mature and become infectious two to three days later. The oocysts of *Cystoisospora* spp. are resistant and remain viable in the environment for months. The infection with these species is mostly self-limiting, which means the treatment of infected host is not necessary, and the most important clinical manifestations of the disease caused by these species, termed cystoisosporiasis, is mild diarrhea; however, it may cause malabsorption syndrome and weight loss, mostly resulting from a severe coccidian infection in the host (5). *Cystoisospora* has also been reported in immunocompetent patients as well as in patients with other cellular immunodeficiencies, such as human T-lymphotropic type 1 infection (6), lymphoblastic leukemia, adult T-cell leukemia, Hodgkin's disease, and non-Hodgkin lymphoma (7). It has also been reported in patients taking immunomodulators such as tumor necrosis factor (TNF)-inhibitors (8). Symptoms of Cystoisosporiasis suggest a toxin-mediated mechanism, yet no toxin has been identified so far (9). In humans, extraintestinal forms of cystoisosporiasis are rare. The gold standard diagnostic method for detection of this parasite species is based on duodenal biopsies and the observation

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of intracellular stages of this parasite (10). In the soil samples and stool of infected hosts; however, concentration methods such as flotation are useful for detection of parasite oocysts (11). Given that the sporulation of oocysts occurs in the environment (11), therefore, this study was designed and was first of its kind to evaluate the prevalence of *Cystoisospora* spp. oocysts in the soil collected from Kermanshah city, west of Iran.

2. Materials and Methods

2.1. Study Area

Kermanshah city is the center of Kermanshah province, located in the west of Iran. According to the 2011 census in Iran, this city had a population of 851405 people. In addition, this city has a moderate climate with a partially cold winter and rainy spring and is bound by the Zagros Mountains.

2.2. Sample Collection

Considering that this study was first of its kind in this region, the sampling method was according to judgmental sampling. The present study was conducted from August to December 2014 in Kermanshah city. For this objective, 192 soil samples were collected from six regions of Kermanshah city including two regions in the city center, center 1, and center 2, as well as one region in each of the east, south, north and west regions. In each region, four public parks and four primary schools were selected, from each of which four soil samples were obtained. Overall, from each region, 32 soil samples were collected, 16 of which were obtained from public parks and 16 other ones from primary schools. The harvested samples were about 200 grams and collected 2 - 5 cm below the soil surface and transferred to the laboratory of parasitology of the Isfahan University of Medical Science.

2.3. Oocysts Isolation

In order to isolate oocysts from the collected soils, the flotation method was used (12). For this objective, initially the soils were air-dried and then passed sieve. Subsequently, 2 g of each soil sample was poured into a separate centrifuge tube. Afterwards, 10 mL of a 0.05% Tween 20 solution was added to each tube and vortexed vigorously. The tubes were centrifuged at 1500 × g for five minutes, the supernatants were then discarded and a sucrose solution with concentration of 1.2 g/mL was added to fill the rest of the volume of the tubes. The tubes were vortexed vigorously and centrifuged again, one cover slip was then put on each tube and let stand for 30 minutes. Subsequently, two separate smears were prepared for each sample, one of which was examined by Lugol's solution and the other was stained with modified Ziehl-Neelsen method.

2.4. Modified Ziehl-Neelsen Staining Method

The smears were fixed by absolute methanol, immersed in carbol-fuchsin solution (Sigma, Inc.) for 15 minutes, rinsed in water, and then decolorized with acid-alcohol solution (99 mL ethanol and 1 mL HCl) for 30 seconds. After rinsing the smears in water again, they were re-stained with 0.25% methylene blue solution (Sigma, Inc.) for 30 seconds. Afterwards, the smears were rinsed, air-dried and examined under light microscope at 1000 × magnification (13).

3. Results and Discussion

Many species of *Cystoisospora* that are excreted by animals such as birds, felines and canines may found in soil. In view of sanitary disposal of feces, the only species infecting human *Cystoisospora* belli is rarely found in the environment.

In the present study, out of 192 soil samples, 15 (7.8 %) were infected with *Cystoisospora* spp. oocysts (Figure 1). In public parks, the highest prevalence of oocysts was observed in the center 1 and the south regions, both with 12.5% prevalence. Moreover, in the primary school regions, it was observed in the center two (18.75 %) region followed by the east and west (both 12.5%) regions. No infection was detected in some of the public park regions including the center 2, the east, and the north. In addition, three regions including the center 1, the south, and the north in relation to the primary schools showed the lowest infection rate (all 6.25%) (Table 1). Several studies have been conducted on soil contamination with coccidian oocysts in different regions.

The results of the study conducted by Papajova et al. (14) showed that coccidian oocysts distributed in the environment by dogs' feces contain about 0.2% of the samples. Another study showed that in Tehran, Iran, the dispersion of *Cystoisospora* spp. oocysts is relatively high, with 18.7% contamination rate in the soil collected from this region (15). In Egypt, one study showed that the prevalence rate of soil contamination with this parasite was 4.3% (16). In a study performed on the soil as well as dogs' feces in Brazil, no *Cystoisospora* spp. was found in the samples (1). Contrary to the last-mentioned study, researchers elsewhere showed that the soil samples collected from the south of Brazil were contaminated with *Cystoisospora* spp. (17).

Likewise, the results of one study from Brazil showed that the soil samples were contaminated with this parasite with a prevalence rate of 2.3% (18) and therefore further

Table 1. The Prevalence of *Cystoisospora* spp. in Soil Samples Collected From Kermanshah City, the West of Iran[a]

Regions	Public Parks		Primary Schools		Total	
	Sample number	Positive	Sample number	Positive	Sample number	Positive
Center one	16	2 (12.5)	16	1 (6.25)	32	3 (9.4)
Center two	16	0 (0)	16	3 (18.75)	32	3 (9.4)
East	16	0 (0)	16	2 (12.5)	32	2 (6.25)
South	16	2 (12.5)	16	1 (6.25)	32	3 (9.4)
North	16	0 (0)	16	1 (6.25)	32	1 (3.1)
West	16	1 (6.25)	16	2 (12.5)	32	3 (9.4)
Total	96	5 (5.2)	96	10 (10.4)	192	15 (7.8)

[a] Values are expressed as No. (%).

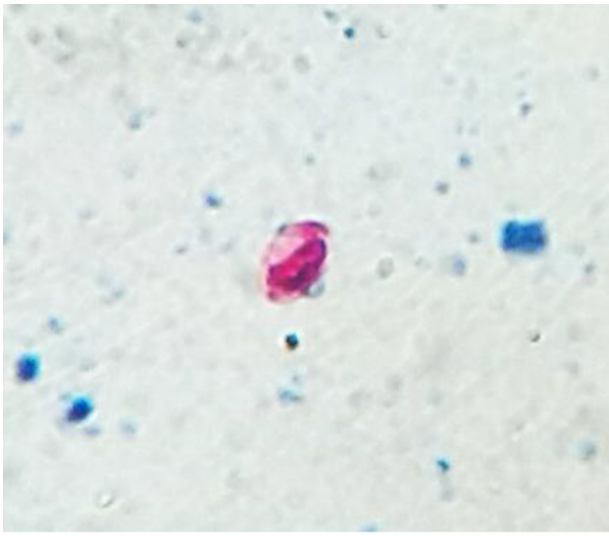

Figure 1. *Cystoisospora* spp. Found in the Soil and Stained With Modified Ziehl-Neelsen Method

studies on this region are necessary. However, the difference among the prevalence rates of this parasite in different countries or different regions of a country is likely due to various geographical climates, host distribution and many other factors (19). In the study performed by Uga et al. (20) in Indonesia, soil contamination with *Cystoisospora felis* was detected. In addition, a study on soil samples in Pula, Croatia, showed no contamination with *Cystoisospora* spp. (21). In Ahvaz city, Iran, the infection of stray cats with *Cystoisospora* spp. was 21.4%, indicating that the environment is also contaminated with this parasite species (22). In Brazil, another study showed that the infection rate of dogs with this parasite was 5.8% (23). In Germany, the prevalence of this parasite in dogs and cats was estimated to be about 22.3% and 21.9%, respectively (24). In Poland, the results of a study showed that *Cystoisospora* spp. oocyst is present in the soil with a low prevalence rate (25). In addition, a survey on soil samples from Turkey revealed that the prevalence of this parasite was 0.2% in this region (26). Martinez-Moreno et al. (27) studied dogs and soil samples in Spain to examine their parasitic burden. The findings of the last-mentioned study revealed that the samples related to both dogs and soil samples were contaminated with *Cystoisospora* spp. oocyst. Another survey conducted by Dubna et al. (28) illustrated that this parasite oocysts are spread in the Czech Republic with a maximum prevalence of 8% in dog feces. This parasite is of public health importance and even by considering all aspects of hygiene principles the eradication of this parasite is usually difficult (29). It should be mentioned that geophagia in malnourished children may occur and therefore they are highly at risk of infection from soil. Some researchers have shown that there is a relationship between socioeconomic situation and parasitic burden in certain regions of Iran (30-33). Overall, the search of the literature indicated that this parasite species might be found all over the world; however, not many studies have been conducted to clarify the status of soil contamination with *Cystoisospora* spp. around the world. In view of what was discussed earlier, environmental factors are very important in the transmission of soil-transmitted parasitic diseases and therefore the infection of animals in a region is not an adequate reason to assume that the soil of the same region is contaminated with identical rates of infection, because depending on the various environmental circumstances, the oocysts survival time can be variable in different regions. The findings of the present study revealed the potential of *Cystoisospora* spp. transmission in the west of Iran, and the contamination of soil in primary schools is higher than public parks. As a result, the identification of *Cystoisospora* species in soil

of this region and in other places is an idea for researchers who work in this field.

4. Conclusion

In view of human infection with *Cystoisospora* spp. and the rise in the number of immune compromised individuals, high contamination of soil with this parasite can be considered as a serious problem in the Kermanshah province. The results of the present study underline soil contamination with *Cystoisospora* spp. as a major public health challenge. Health advancements, public education and improving sanitation situations, especially for deprived people, are the main factors to prevent distribution of this infection. As a result, the findings of the current study could be utilized as a foundation of preventive programs, especially for at risk groups.

Footnote

Authors' Contribution: Study concept and design: Mohammad Ali Mohaghegh and Mohsen Ghomashlooyan; acquisition of data: Mohammad Ali Mohaghegh, Mohsen Ghomashlooyan, Mohammad Reza Vafayi, Zahra Chizari, Roghiyeh Faridnia and Mohammad Falahati; analysis and interpretation of data: Rasool Jafari, Mehdi Azami, and Hamed Kalani; drafting of the manuscript: Hamed Kalani and Mehdi Azami; critical revision of the manuscript for important intellectual content: Mohammad Ali Mohaghegh, Mohsen Ghomashlooyan, Mohammad Reza Vafayi, Zahra Chizari, Roghiyeh Faridnia and Rasool Jafari; statistical analysis: Mohammad Ali Mohaghegh and Hamed Kalani; administrative, technical, and material support: Mohammad Ali Mohaghegh; study supervision, Mohammad Ali Mohaghegh and Hamed Kalani.

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