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Investigation of Soil Contamination With *Cryptosporidium* spp. Oocysts in Different Regions of Yazd, Central Iran

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Abstract

Background: *Cryptosporidium* species are coccidian parasites that cause gastrointestinal disorders in humans and other animals worldwide.

Objective: The aim of this study was to demonstrate the rate of contamination with *Cryptosporidium* spp. oocysts in soils collected from public parks, primary schools, green areas, kindergartens, suburban areas, streets, residential complexes, backyards and a passenger terminal in Yazd, central Iran.

Materials and Methods: This cross-sectional study was conducted from September 2014 to February 2015, and the samples were collected from 9 different study sites and 56 regions. Soil samples were investigated by flotation technique and modified Ziehl–Neelsen staining for *Cryptosporidium* spp. oocysts. Finally, the slides were examined with a light microscope. The data were analyzed using SPSS software version 20.0 and chi-square statistical test.

Results: Of a total of 220 soil samples, 47 (21.36%) were found to contain *Cryptosporidium* spp. oocysts. Statistical analysis showed that there was no significant difference between the contamination rate and different study sites in Yazd, central Iran ($P>0.05$). The highest contamination rate was observed in public parks (38.3%) and the lowest in passenger terminal, kindergartens and streets (4.25%) ($P=0.934$).

Conclusion: The results of the present study show that the contamination of soil with *Cryptosporidium* spp. can be considered a serious problem in Yazd, central Iran. It should be considered particularly in public parks.

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Background

Cryptosporidium as a small coccidian parasite is a threat to public health due to unknown pathogenic mechanisms, the presence of multiple reservoirs, pathogenicity of domestic and wild animals, the creation of dangerous and deadly complications in people with AIDS and transmission from humans to humans.[1] The contamination rates of *Cryptosporidium* spp. in humans and animals are estimated to be more than 4% and more than 9%, respectively.[2] Different clinical symptoms develop from asymptomatic symptoms to severe and fatal parasitic diarrhea. Children and people with AIDS are more likely to be exposed to the dangerous side effects of these coccidia than others[3-5] Sucrose flotation is the best method for isolating *cryptosporidium* oocysts in different specimens (feces, soil, water, rotten materials, etc) and Ziehl-Neelsen staining, Masson's trichrome staining and Giemsa staining for morphological examination of parasites.[5-8]

Materials and Methods

Study Area

Yazd is the driest major city in Iran, with an average annual rainfall of only 60 mm. The city is located about 175 miles southeast of Isfahan and accounts for 6.3% of the entire area of Iran.

Sample Collection

This study is a cross-sectional descriptive study conducted from September 2014 to February 2015, with a total of 220 soil samples collected from public parks, primary schools, green areas, kindergartens, suburban areas, streets, residential complexes, backyards, and one passenger terminal. Each place (for example, a street or a park, etc)

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contains several sites (different parts in each place), from which the samples were collected.

Sample Preparation

Soil samples were investigated by flotation technique for the presence of *Cryptosporidium* oocysts. The slides were stained with a cold modified Ziehl-Neelsen procedure and examined with a light microscope for the presence of *Cryptosporidium* spp. oocysts (Figure 1). The purpose of this study was to demonstrate the rate of contamination with *Cryptosporidium* spp. oocysts in soils collected from public parks, primary schools, green areas, kindergartens, suburban areas, streets, residential complexes, backyards and a passenger terminal in Yazd, central Iran.

Results

A total of 220 soil samples from 9 different study sites were examined, 47 of which (21.36%) contained *Cryptosporidium* spp. oocysts. The highest rate of contamination was detected in public parks followed by primary schools, green areas, kindergartens, suburban areas, streets, residential complexes, backyards and passenger terminal, respectively. The prevalence of soil contamination with *Cryptosporidium* spp. oocysts was shown in (Table 1). Most of the infections were observed in November. The difference was not statistically significant (P=0.796) (Table 2).

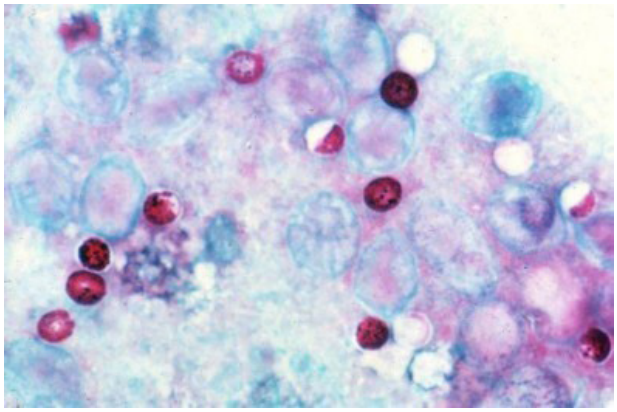

Figure 1. Oocyst of *Cryptosporidium* Spp., Stained by the Modified Ziehl-Neelsen Staining Technique.

Discussion

Soil, water, air, food, infected animals (especially sheep), infected humans (especially children and elderly people) are the main source of human infection.[5, 9] Regarding the importance of the transmission of parasites from animals to humans, studies have been carried out. The prevalence of infection among camel and camel owners in Yazd province (stool examination by Ziehl Neelsen) was 24% and 20.33%, respectively, slightly higher compared to the present study.[24] People with HIV and children are at the highest risk of *cryptosporidiosis*, and studies show the most serious complications in these individuals.[10-12] In studies over several years on human specimens from different individuals in terms of contamination with different species of *Cryptosporidium* in Iran, the prevalence in children, normal people and immunocompromised individuals was 3.65%, 2.94% and 4.54%.[13] A survey on surface water samples in Iran showed that 24 samples (48.98%) of 49 samples were positive for *Cryptosporidium* spp.[14] Regarding Cryptosporidium, the comparison of the results of the present study with those of other studies on different types of human and water samples in Iran showed that the rate of water contamination was much higher than that of soil contamination and soil contamination than human infection. In the present study, a total of 220 soil samples from 9 different study sites were examined for *Cryptosporidium* spp. oocysts by flotation technique and modified Ziehl–Neelsen staining. Forty-seven (21.36%) were found to contain *Cryptosporidium* spp. oocysts and high soil contamination rates (38.3%) were observed in parks so it could be a source of people's contamination. In a study of soil in Isfahan, central Iran, the pollution in more than 22% of soil samples and in more than 60% of public parks contained *cryptosporidium* spp oocysts.[15] In another study of soil in western Iran, out of 192 samples, 49 (25.5%) contained *Cryptosporidium* spp. oocyst, which showed that the contamination rate in public parks and primary schools was 21.9 and 29.2%, and the results of this study showed that soil could be a potential source of cryptosporidiosis.[16] In addition, researchers found that

Table 1. Soil Contamination with *Cryptosporidium* spp. Oocyst in Different Places of Yazd, Ian

Places	Study Sites No. (%)	Study Sites Positive (%)	Soil Samples No. (%)	Positive soil Samples (%)	*P* Value
Parks	16 (28.58)	9 (32.17)	76 (34.56)	18 (38.3)	**0.934**
Primary schools	6 (10.71)	3 (10.71)	26 (11.82)	6 (12.76)	
Green areas	8 (14.29)	3 (10.71)	28 (12.72)	3 (6.38)	
Kindergartens	3 (5.35)	1 (3.57)	9 (4.09)	2 (4.25)	
Suburban areas	5 (8.93)	3 (10.71)	25 (11.36)	5 (10.65)	
Streets	6 (10.71)	2 (7.14)	12 (5.45)	2 (4.25)	
Residential complexes	4 (7.14)	3 (10.71)	16 (7.27)	4 (8.51)	
Backyards	7 (12.5)	3 (10.71)	21 (9.55)	5 (10.65)	
Passenger terminal	1 (1.79)	1 (3.57)	7 (3.18)	2 (4.25)	
Total	56 (100)	28 (100)	220 (100)	47 (100)	

Table 2. The Number of Positive Soil Samples in Terms of Months of the Year

Months of the Year	Total Soil Samples (%)	Positive Soil Samples (%)
September	46 (20.9)	9 (19.15)
October	40 (18.18)	7 (14.89)
November	54 (24.55)	16 (34.04)
December	29 (13.18)	7 (14.89)
January	31 (14.1)	5 (10.65)
February	20 (9.09)	3 (6.38)

P Value = 0.796

42 out of 141 soil specimens (30.5%) were infected with *Cryptosporidium* species in northern Iran.[17] In a study conducted in New York, of 782 soil samples from 37 dairy farms, 133 (17%) were positive for *Cryptosporidium* spp.[18] *Cryptosporidium* species were also detected in 11 (32.4%) of the 34 farm soil samples examined in the Korean peninsula.[19] The results of most studies in Iran (as well as the current study) and the world show that soil contamination varies from 10% to 35% for various *Cryptosporidium* species.[17, 20-25]

Conclusion

The findings of the present study show that soil contamination with *Cryptosporidium* spp should be considered as a serious problem, especially in public parks in our country, and it is recommended that control strategies be developed and implemented to prevent contamination and transmission to humans and other animals. Another point to note is that due to the increased incidence of *Cryptosporidium* oocysts in soil samples, determination of the species of *Cryptosporidium* can play an important role in identifying the sources of soil contamination and, consequently, more accurate decision making on the ways of controlling and preventing them.

Authors' Contributions

Study concept and design: FM, MZ and VH. Acquisition of data: FM, MZ and VH. Analysis and interpretation: MAM.

Ethical Approval

All stages of the plan were carried out in accordance with ethical standards of Shahid Sadoughi University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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