Intestinal protozoa in diarrheic children in an Egyptian rural area: Role of water contamination and other possible risk factors

Original Article

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ABSTRACT

Background: Poor quality of drinking water has been linked to increased prevalence of diarrheal diseases. Intestinal protozoal infections are potential water borne pathogens. They contribute significantly to the burden of diarrheal diseases. Their transmission dynamics vary considerably among geographic areas.

Objective: The present study investigated protozoal infections in diarrheic children and their relation to protozoal contamination of drinking water and other potential risk factors in two Egyptian villages.

Material and Methods: Stool samples of 150 diarrheic children and 40 drinking water samples collected from the houses of participating children were examined for enteric protozoa. Data on potential risk factors for protozoal infection were recorded.

Results: Protozoa were detected in 62.7% of faecal samples and in 32.5% of water samples. Protozoa were not found in water filtered by domestic filters. Protozoal infection was significantly more common among children who were drinking water in which protozoan contaminants were detected (84.6%) compared to those whose water sources were negative (22.2%). Multivariate logistic regression analysis revealed that only two factors were independently linked to intestinal protozoal infection among participating children; neglecting hand wash before eating (adjusted OR: 7.446, 95% CI: 3.113-17.810) and drinking tap water directly without filtration (adjusted OR: 4.653, 95% CI: 1.82-11.895).

Conclusion: The study provides supportive evidence for the role of contaminated water in human infection. Attention should be paid to ensure safe drinking water supply in developing countries. Simple measures such as water filtration coupled with personal hygiene can markedly reduce the risk of infection.

Key Words: Children, diarrhea, drinking water, intestinal protozoa

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INTRODUCTION

Gastrointestinal infections and diarrhea are widespread in developing countries including many tropical regions. Infections of the gastrointestinal tract can be caused by viruses, bacteria, protozoa, helminthes and occasionally fungi[1]. The WHO reported that diarrheal disease affected far more individuals than any other illness, even in regions that include high income countries[2]. Poor quality of drinking water is one of the factors that have been linked to increased prevalence of diarrheal diseases[3].

Intestinal protozoal infections are generally common worldwide. They represent a major cause of diarrheal diseases especially among children in developing countries[1]. *Giardia lamblia* (*G. lamblia*) and *Entamoeba histolytica* (*E. histolytica*) are the most frequently identified protozoan parasites in stool samples. *Cryptosporidium parvum* (*C. parvum*), first described in man in 1976, is now recognized as an important cause of diarrhea in young children and immunocompromized adults[4, 5].

Protozoa are cosmopolitan parasites that are shed in large numbers in the feces of infected hosts, may be found in different water supplies, as environmentally resistant cysts or oocysts[6]. These mentioned pathogenic protozoa, including *Cyclospora cayetanensis* (*C. cayetanensis*) and *Isospora spp* are potential pathogens that have been associated with waterborne infections and water related diarrhea outbreaks[7].

The transmission dynamics of enteric protozoa vary considerably among geographic areas[1]. Understanding the significance of different sources of infection is needed

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to set priorities for control. The present study investigated protozoal infections in diarrheic children and its relation to protozoal contamination of drinking water and other possible risk factors in two Egyptian villages.

MATERIAL AND METHODS

Study design and setting: This cross sectional study was conducted in two Egyptian villages; Abo-Seefa and Kafr-Ziada, located in the Nile Delta in EL-Behera Governorate 88 Km 88 Km from Alexandria. All houses in the two villages have access to piped water supply connected to the local drinking water network. Diarrheic children attending the local rural health units in the period from February to September 2016, were enrolled in the study. The children were all pre-school and school aged (2-10 years) as in this age group they are more susceptible and have the highest rate of morbidity because of intestinal parasites compared with other ages[8,9]. After obtaining an informed consent from parents/guardians of children, an interviewing questionnaire was used to collect demographic and medical data as well as information on factors that might be associated with exposure to protozoal infection and including the domestic source of drinking water whether it was direct tap water or household filtered water. Drinking water samples were collected from the domestic source using clean containers which were labeled immediately after sampling and brought to the laboratory in ice boxes for examination within 24 hrs. Stool samples were collected in labeled clean plastic containers, transported to the laboratory where they were divided into two parts: one was used for saline and iodine wet mounts and the other part was preserved in10 % formalin for concentration and permanent staining[10].

Sample size calculation: Based on 17% infection rate for *Cryptosporidium* and 21% infection rate for *Giardia* reported in earlier studies in Egypt[11,12] and with 10% error, a sample of 150 children (75 from each village) was considered adequate for the study. Water samples were collected from the domestic drinking water source of 40 participating children. This sample size was calculated with 10% error based on *Cryptosporidium* detection rate of 13.7% and *Giardia* detection rate of 11.9 % in water samples examined in a previous study in Egypt[13].

Examination of stool and water samples: Stool samples were examined using saline and iodine wet mounts, formalin ethyl-acatate sedimentation technique and permanent staining with trichrome and modified Ziehl-Neelsen acid fast stains[10]. Water samples (10 liters each) were filtered using cellulose acetate membrane filter with 0.8 um pore size. The filter was rinsed with one liter of de-ionized water containing 0.1% tween 80 elute buffer solution and the resulting suspension was washed and concentrated by centrifugation[14]. The sediment was examined by saline and iodine wet mounts as well as trichrome and modified Ziehl-Neelsen stains.

Statistical analysis: Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Comparison of frequencies among groups was performed using Chi-square or Fisher's exact test. A *P* value less than 0.05 was considered statistically significant. Univariate analysis was used to identify variables associated with protozoal infection among diarrheic children. Odds ratio (OR) and 95% confidence interval (CI) were calculated to determine the ratio of the odds of an event in one risk group to the odds in the non- risk group. Multivariate logistic regression analysis was performed to calculate adjusted OR and to identify independent risk factors where all variables that were significant in the univariate analysis were included in the model.

The protocol of the study was approved by the Research Ethics Committee of the Medical Research Institute (MRI), Alexandria University.

Ethical consideration: An informed consent was obtained from parents/guardians of the enrolled children. The protocol of the study was approved by the Research Ethics Committee of the Medical Research Institute (MRI), Alexandria University.

RESULTS

In Abo-Seefa village, the mean age of participating children was 6.49 ± 2.88 years and 64% were males. In Kafr-Ziada, the mean age was 6.24 ± 3.15 years and males constituted 44/75 (58.7%) of the sample. The overall percentage of parasitic infection among participating children was 94/150 (62.7%) *G. lamblia* was the most prevalent, 35/150 (23.3%), followed by *Blastocystis* spp., 17/150 (18.0%). Less common protozoa were *Cryptosporidium* spp. and *Entamoeba* complex (*histolytica/dispar/moshkovskii*), 16/150 (10.6%) each. The non-pathogenic *Entamoeba coli* (*E. coli*) was found in 9/150 (6%) children. The least common protozoan parasites were *Cyclospora* spp. and *Dientamoeba fragilis* (*D. fragilis*) (Table 1). Helminthic parasites were less frequently encountered and all helminthes infected children had simultaneous protozoan infection. The overall percentage of parasitic infection was significantly higher in Kafr-Ziada village (72%) compared to Abo-Saeefa village (53.3%), (Table 1).

Protozoan contaminants were detected in 13/40 (32.5%) drinking water samples from the two villages. *Cryptosporidium* spp. and *C. cayetanensis* were the most frequent (4/40; 10%), followed by *Entamoeba* complex (3/40; 7.5%). *Blastocystis* spp., *G. lamblia* and *E. coli* were also identified, (1/40; 2.5%). There was no statistically significant difference between the two villages regarding the percentages of different protozoa in water samples (Table 2). All contaminants were found in the samples taken directly from taps (34/40; 85%) but not in filtered tap water (6/40; 15%).

Protozoal infection was significantly more common in children drinking water contaminated with protozoa (11/13; 84.6%) compared to children whose water sources were negative (6/27; 22.2%). Protozoal contamination of water was associated with 19.25 higher risk of protozoal infection in diarrheic children (95% CI: 3.316-111.747), (Table 3).

Univariate analysis of variables associated with intestinal protozoal infection among 150 diarrheic children revealed that four factors were significantly associated with higher risk of infection including residence in Kafr-Ziada village (OR:2.25, 95% CI: 1.142-4.433), neglecting hand wash before eating (OR: 7.754, 95% CI: 3.479-17.285), contact with animals (OR: 2.51, 95% CI:1.266-5.14) and drinking unfiltered tap water (OR: 4.673, 95% CI:2.122-10.29). On the other hand, the child age and gender, attending day care centers and exposure to canal water were not associated with increased risk of infection (Figure 1). In the multivariate model (Figure 2), only two factors were independently linked to intestinal protozoal infection; neglecting hand wash before eating (adjusted OR: 7.446, 95% CI: 3.113-17.810) and drinking tap water directly without filtration (adjusted OR: 4.653, 95% CI: 1.82-11.895).

Table (1): Parasitic infections in the studied diarrheic children

Parasitic infection	Village						*P* values
	Abo-Seefa (n=75)		Kafr-Ziada (n=75)		Total (n=150)		
	No	%	No	%	No	%	
No infection	35	46.6	21	28.1	56	37.3	
Overall infection **	40	53.3	54	72.0	94	62.7	0.018*
Total	75	50%	75	50%	150	100%	
Protozoa							
G. lamblia	15	20.0	20	26.6	35	23.3	0.334
Entamoeba complex	6	8.0	10	13.3	16	10.6	0.290
***Blastocystis* spp.**	11	14.6	16	21.3	27	18.0	0.288
D. fragilis	0	0.0	1	1.3	1	0.6	1.000
C. cayetanensis	0	0.0	2	2.6	2	1.3	0.497
***Cryptosporidium* spp.**	7	9.3	9	12.0	16	10.6	0.597
E. coli	6	8.0	3	4.0	9	6.0	0.494
Total protozoal infections	45	60	61	81.3	106	70.7	
Helminths							
E. vermicularis	0	0.0	1	1.3	1	0.6	0.497
H. nana	1	1.3	1	1.3	2	1.3	1.000
A. lumbricoides	2	2.6	2	2.6	4	2.6	1.000
***Ancylostoma* spp.**	1	1.3	1	1.3	2	1.3	1.000
S. mansoni	0	0.0	1	1.3	1	0.6	0.497
Total helminthes infections	4	5.4	6	8	10	6.7	

*Statistically significant (*P*< 0.05)

**Some children were infected with more than one parasite

Table 2: Protozoan contaminants in drinking water samples.

Intestinal protozoa	Village: Abo-Saeefa (n=20) No.	%	Zafr-Ziada (n=20) No.	%	Total (n=40) No.	%	*P values*
Total	7	35	6	30	13	32.5	0.736
***Entamoeba* complex**	2	10	1	5	3	7.5	1.000
G. *lamblia*	0	0	1	5	1	2.5	1.000
***Blastocystis* spp.**	0	0	1	5	1	2.5	1.000
C*. cayetanensis*	2	10	2	10	4	10	1.000
***Cryptosporidium* spp.**	3	15	1	5	4	10	0.620
E. coli	0	0	1	5	1	2.5	1.000

E. coli and *Cryptosporidium* spp. were detected simultaneously in one water sample in Kafr-Ziada

Table 3: Protozoal contamination of drinking water samples and the corresponding infection in diarrheic children

Water contamination	Protozoal infection: Positive No.	%	Negative No.	%	Total No.	*P* values	OR	95 % CI LL	UL
Positive	11	84.6	2	15. 4	13	<0.001*	19.25	3.316	111.747
Negative^R	6	22.2	21	77.8	27				
Total	17	42.5	23	57.5	40				

OR: Odds ratio, CI: Confidence interval, LL: Lower limit, UL: Upper Limit,
R: Reference group *: Statistically significant at $P \leq 0.05$.

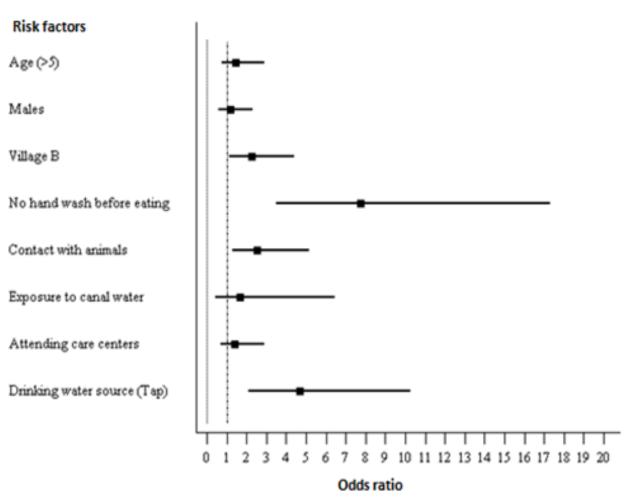

Fig. 1: Forest plot showing univariate analysis of risk factors for protozoal infections among 150 diarrheic children in Abo-Seefa and Kafr-Ziada villages, EL-Behera Governorate.

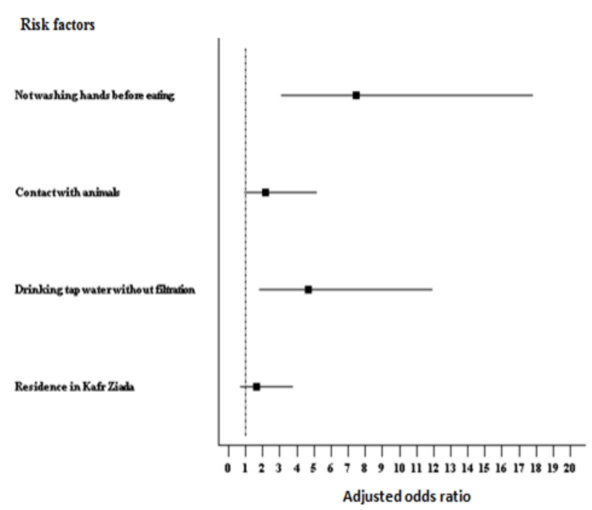

Fig. 2: Forest plot showing logistic regression analysis of risk factors for protozoal infection among 150 diarrheic children in Abo-Seefa and Kafr-Ziada villages, EL-Behera Governorate.

DISCUSSION

Enteric protozoa are recognized as an important cause of diarrhea in children. They are potential water borne pathogens that may be found in water bodies due to unsanitary fecal contamination[15]. In the current study, 62.7% of diarrheic children were infected with one or more intestinal protozoa. *G. lamblia* was the most common, followed by *Blastocystis, Entamoeba* complex and *Cryptosporidium* spp. This finding is consistent with previous studies in Egypt confirming an unchanging pattern of intestinal parasitic infection. Mousa *et al*. in Great Cairo, examined a total of 110 diarrheic stool samples for parasitic causes and found that the overall percentage of parasitic infection was 60.9%[16]. In a study conducted in Damanhur city, El-Behera Governorate, the percentage of parasitic infection among five hundred preschool children was 51.8%[17].

G. lamblia is the most commonly isolated intestinal parasite throughout the world. Rates of 20 - 40% were reported in developing countries, especially in children. An estimated 10% of the world's population is infected with *E. histolytica*; the highest prevalence is in developing countries with the lowest levels of sanitation[18].

As with the present study, Firdu *et al*. found that the prevalence rates of *G. lamblia*, *Cryptosporidium* spp, and Entamoeba complex among children of ages under 13 years with diarrhea in Ethiopia were 15.65%, 9.56%, and 4.35% respectively[19]. El-Helaly *et al*. reported that the percentage of *Cryptosporidium* infection among children in the age group from 1 to 5 years in Cairo was 11.3%[20].

Other studies recorded higher infection rates of different parasites. Koffi *et al*., in central Côte d'Ivoire reported that the species specific prevalence among children under five years was 36.93% for *C. parvum* and 22.55% for *E. histolytica*[21]. Idris *et al*. reported that *Blastocystis* spp. comprised the largest proportion of parasites (96%) identified in diarrheic children mostly aged one to five years in Indonesia[22]. These variations in the percentages of parasitic infection may reflect difference in place, socioeconomic, and environmental housing conditions, education and personal hygienic care.

In the present study, enteric waterborne protozoa were identified in 13/40 (32.5%) of the collected tap water samples with the coccidian parasites, *Cryptosporidium* spp. and *C. cayetanensis* being the most frequently isolated, 4/40 (10%) each; followed by *Entamoeba* complex 3/40 (7.5%). *G. lamblia, Blastocystis* spp. and *E. coli* were also identified , each in one sample (2.5%).

Cryptosporidium is more resistant than other pathogens to disinfectants[23]. It is not easily removed by physical processes due to its small size and there is some evidence that it survives longer than other protozoa in water environments. It has been used as a reference pathogen for risk assessment of drinking water contamination by protozoan pathogens where its elimination ensures control of all other similar protozoa of concern[15].

In Egypt, protozoal contamination of drinking water was previously reported with variable rates. In northern part of El-Minia Governorate, out of 48 tap water samples, six (12.5%) were contaminated with *Cryptosporidium* oocysts[24]. In El-Ismailia city, only two out of 64 tap water samples from four districts were positive for *Cryptosporidium* oocysts with a mean concentration of 25 oocysts/L. Positive samples were found in only one district suggesting that contamination may have occurred due to leakage of oocysts into the distribution system after treatment[25].

In a recent study in Gharbiya Governorate, of 36 tap water samples examined for protozoan contaminants, *Giardia* cysts were found in eight samples and *Cryptosporidium* oocysts in 14 samples[12]. In a large survey involving 840 potable water samples from seven districts in Dakahlia Governorate, *Cryptosporidium* spp. and *G. lamblia* were the most common disease-agents (3.1% and 2.1% respectively). Other protozoan contaminants were *E. histolytica* (1%), *B. hominis* (1%), *Iodamoeba* spp., (0.5%), *Isospora belli* (0.47%), *E. coli* (0.36%) and *C. cayetanensis* (0.24%)[26]. In another study in Fayoum Governorate, 95 water samples were collected from different water sources, taps (65) and tanks (30), from 6 districts. All water samples collected from tanks (100%) were contaminated by protozoa of medical importance, while only 6 (9.2%) of the tap water samples were pathogen free. The overall detection rates of contaminants in water sources were 86.3%, 52.6%, 13.7% by free living amoeba, *Cryptosporidium* spp. and *G. lamblia*, respectively[27].

In the current studied villages, it was observed that storage of water in roof tanks was used in all houses to overcome intermittence of water supply. Contamination of water may have occurred as a result of inappropriate storage inside the dwellings. Storage of water increases the risk of contamination and reduces the disinfectant residual[28, 29]. However, shortcomings in water treatment and unsatisfactory disinfection at the water treatment plant cannot be excluded.

The ability of enteric protozoa to cause illness even with low infective dose makes protozoal contamination of drinking water a potential public health threat[15, 30]. The present study revealed a statistically significant association between the detection of protozoan infection in diarrheic children and protozoan contamination of their domestic source of water confirming the role of water in transmission of intestinal protozoa. This finding is consistent with those of Nimri who found *Cryptosporidium* and *Cyclospora* oocysts in water storage tanks at homes of six patients who were infected by these parasites but not in tank water of non-infected patients[31]. In a study conducted in a rural area in Zimbabwe where drinking water is obtained from wells, boreholes or springs and is mostly consumed untreated, high 83% prevalence of intestinal protozoan infections was reported among the study subjects, and protozoan species were identified microscopically in 11/30 (36.6%) water samples examined[32].

In the present study, none of the samples collected after domestic filtration were contaminated by protozoa; while drinking tap water directly, without using filters, was identified as an independent risk factor for protozoal infection in diarrheic children Using household filters has been shown to be a practical and cost effective method for prevention of diarrheal diseases[15, 32]. In a study in Iran, there was no parasitic contamination in three samples from water-selling stations probably due to appropriate filtration[10]. Selecting the most appropriate filter pore size is crucial; a size of one µm or less for the filter media pore is recommended to ensure removal of *Cryptosporidium* oocysts from water[15]. In the present study, it was not possible to record the filter pore size since most users were unaware of the detailed properties of their household filters.

Another variable independently associated with protozoal infection in the current work was neglecting hand wash before eating. Similar results were reported by EL-Masry *et al*.[33] who showed that hand washing before meals was the most important factor of personal hygienic habits that has significant effect on the rate of intestinal parasitic infections. Mohammad *et al*.[34] reported that improper hygiene in food handling was a significant risk factor for parasitic infection. Another study by Al-Mekhlafi *et al*.[35] found that the practices of not washing hands before eating and not clipping fingernails were significantly associated with a higher likelihood of infections among schoolchildren.

In the present work, a higher percentage of infection was detected among those who were residing in Kafr-Ziada compared to Abo-Seefa village. Though not confirmed in the multivariate model, this may be linked to the low socioeconomic level, lack of sanitation and bad personal hygiene in Kafr-Ziada. On the other hand, Abo-Seefa is characterized by a higher standard of living, better health awareness and availability of medical services.

Some protozoal infections are known to be zoonotically transmitted. The dynamics of zoonotic transmission differ from one geographic area to another[36]. Our results revealed that contact with animals was significantly associated with increased risk of protozoal infection in the univariate but not in the multivariate model. Other authors reported that residence in rural areas, contact with animals and poor hygiene are independent variables linked to intestinal parasitosis[16,17].

In the present work, the child age and gender were not associated with increased risk of protozoal infection. This is possibly because of sharing the same environmental and social conditions. While older age groups may have greater exposure due to outdoor feeding and unhygienic toilet practices with less parental supervision, younger children are more vulnerable due to lower immunity[37, 38]. Males may be more exposed to infection sources as a result of their habits related to playing and other outdoor activities[39].

Close contact of children in day care centers favors anthroponotic transmission of most enteric protozoa unless good hygiene is practiced[40,41]. Lack of significant association with protozoal infection in the present study suggests that person to person spread plays a limited role compared to waterborne transmission.

CONCLUSION

Intestinal protozoal infections predominate among rural diarrheic children, constituting a greater burden than helminthic infections. The observed association between contamination of drinking water by protozoa and their detection in diarrheic children provides a supportive evidence for the role of contaminated water in human infection. Attention should be paid to ensure safe drinking water supply in developing countries. Simple measures such as water filtration coupled with personal hygiene can markedly reduce the risk of infection.

Author contribution: NF AbdEllatif participated in the conception and implementation of work, interpretation of the results, and writing of the manuscript. MA Mohamed conceived and designed the experiments; reviewed the presentation and interpretation of the results. HA El-Taweel participated in the design, conception and implementation of work, interpretation of the results, and writing of the manuscript. MH Hamam collected the stool samples and participated in the implementation of the work. MN Saudi reviewed the presentation and interpretation of the results.

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