Journal of the Egyptian Society of Parasitology, Vol.44, No.3, December 2014

J. Egypt. Soc. Parasitol. (JESP), 44(3), 2014: 559 - 566

PRESENT STATUS OF PROTOZOAN PATHOGENS CAUSING WATER-BORNE DISEASE IN NORTHERN PART OF EL-MINIA GOVERNORATE, EGYPT

By

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Abstract

Little is known about the role of different water supplies in the diversity and public health significance of pathogenic protozoan parasites. Most of these organisms have been ubiquitous in waters worldwide. The numbers of waterborne infections indicate a significant risk for their transmission even by drinking water. Hence, a total of 336 water samples were collected during 2009-2013 from different water sources from different areas of northern part of El-Minia Governorate, Egypt and were investigated for pathogenic protozoa. They were examined by direct microscopy followed by Modified Ziehl-Neelsen and Giemsa stains. 140 samples (41.7 %) were positive (statistically significant; P value P<0.0003). Prevalence rates were in Summer (66.7%), Spring (51.1%), Fall (26.2 %) and Winter (22.6%). These data were statistically significant (P<0.0001). The commonest protozoa detected as a single infection was *Cryptosporidium* sp. (53.17%) found in all water supplies, followed by *Blastocystis hominis* (15.87%)*, Cyclospora caytenensis* (11.9%)*, Entamoeba histolytica/ dispar* (8.73%) *Giardia lamblia* (6.35%) and *Naegleria* sp.*,* (3.97%). Moreover, there were 14 samples with mixed parsitic infection: they were *Cryptosporidium* sp. and *B. hominis* in six samples*, Cryptosporidium* sp. and *C. caytenensisin* five samples and *Cryptosporidium* sp. and *E. histolytica/dispar* in three samples. The most common contaminated water source was ponds where 32 samples (66.7%) were positive followed by canal water 30 samples (62.5%).

The results were discussed and the recommendations were offered.

Key words: Water samples, pathogenic protozoa, El- Minia Governorate.

Introduction

Water plays an important role in the transfer of many pathogenic microorganisms. Waterborne diseases are transmitted through contact with or consumption of infected water. Microorganisms that cause waterborne diseases are viruses, bacteria, protozoa, and helminthes. In Egypt, waterborne diseases represent a public health problem as the majority of rural population obtains their water supply from unprotected streams and ground water. Moreover, the control of waterborne diseases transmission is actually difficult, as certain pathogens, such as *Cryptosporidium*, are highly resistant to many traditional processes used for disinfection of water. In this study we will stress on protozoan parasites as one of the most important causes of the water-borne diseases. Some protozoan parasites such as *Cryptosporidium* sp., *Cyclospora cayetanensis,* and *Giardia lamblia* were responsible for emerging cases of contaminated water (Céline *et al,* 2009). More than 160 waterborne outbreaks of cryptosporidiosis were reported worldwide in the last ten years (Carmena *et al,* 2006). The first *C. cayetanensis* waterborne outbreak was described in 1990 (Ortega and Sanchez, 2010). WHO (2011) confirmed the detection of *C. cayetanensis*, *Microsporidia*, *Isospora belli* and *C. parvum* in drinking water. Besides, *Giardia* is one of the commonest causes of waterborne diseases for over 30 years (Briancesco and Bonadonna, 2005). Additionally, cases of acanthamoebic keratitis have been reported due to use of tap water in preparing solutions for washing contact lenses. In El-Minia Governorate the percentage of such pathogenic protozoa has been documented in humans (Abdel-Hafeez *et al*, 2012). However, very few epidemiological surveys have been conducted on the occurrence of these protozoa in water used for human consumption in this Governorate. Therefore, this paper aims to outline the prevalence of the pathogenic protozoan parasites that cause waterborne infection in the northern part of El-Minia Governorate, Egypt, as a pioneer milestone in the future plans for the provision of clean water supply for the community; which is the target of many recent studies (Anuar *et al,* 2013).

Materials and methods

Collection of water samples: The present work is a longitudinal descriptive study conducted in the northern part of El-Minia Governorate, in Upper Egypt, 234 Km south to Cairo. A total of 336 samples of water have been collected from six cities of the northern part of El-Minia Governorate (Tab.3a). Samples were collected from seven different types of water (River Nile, waterworks, tap water, water pumps, water tanks, ponds and canal water) from each city. Eight samples were collected from each source, two samples in each season; Spring (May), Summer (August), Fall (October) and Winter (January) of 2009-2013 (56 samples from each city) to avoid seasonal fluctuations in oocyst contamination.

Sample processing: Each water sample was 10 ml in volume. Samples were transmitted immediately after collection to the laboratory of Parasitology Department, Faculty of Medicine, El-Minia University for examination. Water samples were processed within 12 hours after collection.

Filtration method: Water samples were subjected to filtration using membrane filter dissolution method (Aldom and Chagla, 1995). They were filtered through a mesh sieve to remove the large particles and then concentrated by filtration on cellulose- acetate filters (0.8μm pore size, Nuclepore Whatman, NJ, USA). The filtrate was put in 15 ml centrifuge tubes and centrifuged at a speed of 3000 g for 10 min. The supernatant was decanted and the pellets were subjected to the following: 1) Direct microscopic examination through saline and iodine wet mount smears. 2) Fixation and smear preparations where pellets were mixed with 10 ml of fixation buffer (sodium-acetate acetic acid formalin, SAF) and incubated for 1h to be fixed. The suspension was centrifuged at 2,000 g for 5 min. Two smears were dried in air, fixed with methanol, stained by Modified Ziehl-Neelsen stain) (El Shazly *et al,* 2006) and Giemsa stain (Garcia, 2001).

Statistical analysis: Statistical Package of SPSS version 16 for windows was used. Descriptive statistics were calculated. For qualitative data, $\chi 2$-test and Z test were used for proportions. A significant *P*-value was considered when it was less than 0.05.

Results

The results are illustrated in tables (1-5)

Sampling places and the number of the positive samples for parasites: One hundred and forty samples (41.7%) out of 336 samples were diagnosed positive for parasitic infections. It has been found that Beni-Mazar City had highest rate of contamination (58.9%) while El-Minia City had the lowest rate (21.4 %). These data were statistically significant (P value <0.0003).

Seasonal variations of parasitic infections at different periods of year: Fifty six (66.7%) samples were diagnosed positive in summer, 43 (51.1%) samples were positive in spring. On the other hand, 22 (26.2 %) samples were diagnosed positive in fall and 19 (22.6%) samples were diagnosed positive in winter (Tab. 2). These results were statistically significant (P<0.0001).

The prevalence of protozoan parasites detected is shown in (Tabs.1, 3a, b). The most common protozoa detected as a single infection was *Cryptosporidium* sp. (53.17%), followed by *Blastocystis* sp. (15.87%), *Cyclospora caytenensis* (11.9%), *E. histolytica/ dispar* (8.73%), *Giardia lamblia* (6.35%) and *Naegleria* sp, (3.97%).

There were 14 samples with mixed parasitic infection: *Cryptosporidium* sp. and *Blastocystis* sp. in six samples, *Cryptosporidium* sp. and *C. caytenensis* in five samples and *Cryptosporidium* sp. and *E. histolytica/ dispar* in three samples

Relation between the type of protozoan parasites detected and the type of water collected: Prevalence of protozoan parasites detected from different water sources is shown (Tab. 4). The commonest water source contaminated with protozoan parasites was ponds where 32 samples (66.7%) were positive followed by canal water 30 samples (62.5%). These results were statistically significant (P< 0.0001). The prevalence of the protozoan parasites detected from the different sources of water is shown in (Tab.5) which illustrates that *Cryptosporidium* sp. could be detected from all water sources.

Discussion

Lack of access to healthy water is one of the most important problems that the majority of the world's population is facing. Contamination of drinking water with protozoan pathogens threats millions of people in developing world (WHO, 2011). The problem is particularly seen in rural Egyptian villages (Khairy *et al,* 1982). The main source of water in Egypt is the Nile River and this water is affected by many polluting activities such as sewage and industrial discharge, human activities and run-off from agricultural fields (El-Shazly *et al,* 2007). Water-borne outbreaks caused by *Cryptosporidium* sp., *C. caytenensis*, *B. hominis* and *G. lamblia* have been documented not only from Egypt but also worldwide even in developed countries (Dolejs *et al,* 2000 in Czech drinking water, Tsushima *et al,* 2001; Hashimoto *et al,* 2002 in Japan, Briancesco and Bonadonna, 2005 in Italy; Karanis *et al,* 2006 in Russia and Bulgaria, Céline *et al,* 2009 in France).

In the present study, the most common protozoa detected in different types of water as a single infection was *Cryptosporidium* sp. (47.8%)*,* followed by *Blastocystis* sp. (14.3%)*, C. caytenensis* (10.7%)*, E. histolytica/dispar* (7.9%), *G. lamblia* (5.8%) and *Naegleria* sp. (3.6%). Moreover, there were 14 samples with mixed infections: *Cryptosporidium* sp. and *Blastocystis* sp. in six samples (4.3%)*, Cryptosporidium* sp. and *C. caytenensis* in five samples (3.6%)*,* and *Cryptosporidium* sp. and *E. histolytica/dispar* in three samples (2.1%)

In this study, *Cryptosporidium* sp. oocysts were consistently detected in almost all types of water samples collected. The high occurrence of *Cryptosporidium* sp. in the water sources can be explained by the oocysts being resistant to disinfection. Moreover, *Cryptosporidium* oocysts are not inactivated by chlorination practices generally applied in the production of drinking-water. This result matched with other studies done by Carmena *et al.* (2006); El Shazly *et al.* (2007). In this connection, Chauret *et al.* (2001) recommended Chlorine dioxide for better cryptosporidial inactivation in water. In Egypt, sewage is subjected to minimal treatment and effluent is discharged into Nile River, lakes and seas (El Shazly *et al,* 2007). Reservoir animal hosts may be of utmost importance in contaminating drinking waters in Egypt. Thus, El-Khodery and Osman (2008) found 14.9% of buffalo calves less than 3 months age were infected with *Cryptosporidium* spp. in middle Egypt, El-Madawy *et al.* (2010) detected cryptosporidial infection among stray dogs in El-Behira Province and pointed out to the existence of a genotype that may play an important role as a source of human and farm animal cryptosporidiosis through drinking water contamination, Hassanain *et al.* (2011) illustrated the high prevalence of *Cryptosporidium parvum* in calves in El-Behira Province and pointed out to the possible zoonotic transmission between calves and humans in that region, Shaaban *et al.* (2011) reported the occurrence of cryptosporidiosis in native Egyptian quails and recommended fecal control of these birds and Amer *et al.* (2013a,b) discussed the public health potential of *Cryptosporidium* spp. in Egyptian water buffaloes and concluded that the commonly occurrence of *C. parvum* IId subtype in both buffalo calves and humans highlights the potential role of zoonotic transmission in the epidemiology of cryptosporidiosis. Moreover, Fayer *et al.* (2006) demonstrated the risk of human beings cryptosporidial infection through drinking water contaminated by the feces of youg diary cattle in USA. Therefore, future studies are recommended to determine the risk of zoonosis in water-borne infections but this also requires the assessment of the viability and infectivity of oocysts detected and the use of molecular methods which add value to performance-based morphologic methods; as many forms detected in water may originate from species/genotypes that are not infectious to humans (Quintero-Betancourt *et al,* 2003; Weintraub, 2006; Smith and Nichols, 2010). El-Temsahy *et al.* (2014) in Alexandria proved that cryptosporidia and cyclosporidia of freshwater fishes are not infectious to humans.

In this study, six tap water samples were contaminated with cryptosporidial oocysts, (12.5 %) out of 48 samples which are in accordance with observations by several studies; in Egypt (El Shazly *et al*, 2007) and in Spain (Carmena *et al*, 2006). In controversy, Nishi and others could not detect any *Cryptosporidium* in public treated water of Bolisista, Brazil (Nishi *et al.,* 2009 a,b).

Blastocystis sp. was also detected in 16.9 % of the water samples collected. This finding was in agreement with the data obtained by Leelayoova *et al.* (2008). They found that *Blastocystis* prevalence was 18.9% in school children of a rural community in Thailand and this infection came from drinking water. Waterborne transmission of blastocystosis had been suggested by a number of epidemiologic studies (Suresh *et al*, 2005; Li *et al*, 2007; Leelayoova *et al*, 2008). Furthermore, Ithoi *et al.* (2011) reported the occurrence of *Blastocystis* in two rivers located in recreational areas in Malaysia. Yan *et al.* (2007) indicated molecular-based evidence supporting the zoonotic potential of water-borne transmission of *Blastocystis* sp. subtype 5 from pigs and Lee *et al.* (2012) reported *Blastocysis* sp. subtype 4 in buffalos and pigs in Nepal and blamed it as a water-borne zoonosis for human blastocystosis.

In the present work, *C. cayetanensis* was detected in 13.0%, which concurred with Nimri (2003) in Jordan who reported that its oocysts were detected in the sediment of water in home storage tanks of patients with diarrhea. Water-borne *C. caytenensis* transmission was reported by few studies (Ortega and Sanchez 2010; Ithoi *et al*, 2011, WHO, 2011).

Giardiasis has been associated with drinking-water supplies over the past 30 years (Briancesco and Bonadonna, 2005) as the commonest cause of waterborne outbreaks since its cysts are more resistant to chlorine treatment, but not as resistant as *Cryptosporidium* oocysts (WHO, 2011). *G. lamblia* (5.2 %) was detected but lower than *Cryptosporidium* spp. (52.6%). This result mismatched with that obtained in some abroad countries by (Briancesco and Bonadonna, 2005; Hörman *et al*, 2004). Bednarska *et al.* (1998) found 14% of calves infected with *Giardia* sp. and stressed their role as reservoirs for human infection..

In the current study, *E. histolytica/dispar* (9.0 %) was detected in water samples from fecally contaminated ponds and canal water where water is untreated and fecally contaminated. Transmission of amoebiasis by water is common in developing countries (El-Szhazly *et al.,* 2007). Moreover, Ximénez *et al*. (2011) stated that the asymptomatic cyst passers and the intestinal amoebiasis patients are the transmitters; they excrete cysts in their feces, which can contaminate food and water sources.

In Egypt, the prevalence of *Cryptosporidium* sp. and *G. lamblia* positive water samples was reported in El-Gharbia Governorate (Antonios *et al*, 2001), in Abo-El Nomros and El Hawamdia, Giza Governorate (Ali *et al,* 2004), in El-Dakahlia Governorate (El Shazly *et al*, 2007), and in Ismailia Governorate (Rayan *et al,* 2009) and in Alexandria

Governorate (Khalifa *et al,* 2011). This is not surprising as these protozoan parasites are usually connected with poor sanitation. Contaminated water passes freely through water treatment plants because cryptosporidial oocysts in particular are not readily killed by chlorine and filtration may be ineffectual or non-existant.

In the present study, seasonal distribution of protozoan infection proved to be significantly highest in summer (66.7%); these results agreed with Keeley and Faulkner (2008). It is obvious that hotness, humidity and stagnation of water increase the incidence of parasites in water. Seasonal peaks of human infection with *Cryptosporidium* have been reported in North America in Spring or late Summer, in Australia during the Summer, in Germany in late Summer, and in Central America during the rainy season. Hashimoto *et al.* (2002) advised close management of water supplies in the winter when the temperature is low and Keeley and Faulkner (2008) reported peaks of protozoan water contamination in Spring and late Autumn or early Winter in the United Kingdom and Ireland.

Conclusion

The present work exhibited the update prevalence of pathogenic protozoa in different water supplies in northern part of El-Minia Governorate as a pioneer milestone in the future plans for the provision of clean water supply for the community. The presence of cryptosporidial oocysts in all water supplies; even tap drinking water is hazardous and this may be due to its being least resistant to regular water disinfection practice and this represents a potential risk to public health and water industries. The poor microbial quality of the water supplies in El-Minia Governorate may be due to improper operational skills and management of the various water treatment plants. Hence, an additional water treatment steps are recommended in order to reduce waterborne diseases. Future studies are recommended to determine the risk of zoonosis in waterborne infections. This also requires the assessment of the viability and infectivity of protozoa detected and the use of molecular methods which add value to performance-based morphologic methods; as many forms detected in water may originate from species/genotypes that are not infectious to humans.

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Table 1: Protozoan parasites detected in northern part of El-Minia Governorate

Single Protozoan parasites	No.	%	Z	P value
Cryptosporidium spp.	67	53.17	07	0.2
Blastocystis hominis	20	15.87	10.4	0.0001
Cyclospora caytenensis	15	11.9	12.2	0.0001
E. histolytica/dispar	11	8.73	14.4	0.0001
Giardia lamblia	8	6.35	20.1	0.0001
Acanthamoeba sp.	5	3.97	26.4	0.0001
Total	126	100		

Chi2= 17.8, DF= 5, P< 0.0003

Table 2: Seasonal variations of water sources protozoan contamination

Period of the year	Positive		Negative		Total	
	N	%	N	%	N	%
Summer	56	66.7	28	33.3	84	100
Spring	43	51.1	41	48.8	84	100
Fall	22	26.2	62	73.8	84	100
winter	19	22.6	65	77.3	84	100
Total	140	41.7	196	58.3	336	100

Chi2= 45.5, DF= 3, P<0.0001

Table 3a: Single Protozoa detected in different Northern parts of El-Minia Governorate.

Protozoan parasites.	R. Nile No. %	W. works No. %	Tap No. %	Pumps No. %	Tanks No. %	Ponds No. %	Canal No. %	No. %
Cryptosporidium sp.	15 18.5	10 12.4	6 7.4	9 11.1	12 14.8	17 20.9	12 14.8	81 100
B. hominis	6 23.1	4 15.4	0 0.0	0 0.0	4 15.4	7 26.9	5 19.2	26 100
C. caytenensis	3 15.0	3 15. 0	0 0.0	1 5.0	4 20.0	5 25.0	4 20.0	20 100
E. histolytica/dispar	2 14.3	0 00.0	0 0.0	0 0.0	1 07.1	5 35.7	6 42.9	14 100
G. lamblia	3 37.5	0 00.0	0 0.0	0 0.0	0 00.0	2 25.0	3 37.5	8 100
Acanthamoeba sp.	0 00.0	0 00.0	0 0.0	0 0.0	0 00.0	2 20.0	3 60.0	5 100
Total	29 18.82	17 11.04	7 4.55	106.49	21 13.64	37 24.03	33 21.43	154 100

Table 3b: Mixed Protozoan parasites detected in northern parts of El-Minia Governorate.

Grand total	No. (140)	% (100%)	Z	P-value
Total single	126	90%	15.7	0.0001
Total mixed	14	10%		

Table 4: Prevalence of pathogenic protozoan parasites in different water sources

Type of water examined	Positive		Negative		Total	
	No.	%	No.	%	No.	%
River Nile	26	54.2	22	45.8	48	100
Waterworks	16	33.3	32	66.7	48	100
Tap water	6	12.5	42	87.5	48	100
Pumps water	10	20.8	38	79.2	48	100
Tanks water	20	41.7	28	58.3	48	100
Ponds	32	66.7	16	33.3	48	100
Canal water	30	62.5	18	37.5	48	100
Total	140	41.7	196	58.3	336	100

Chi^2= 50.7, DF=6, P< 0.0001

Table 5: Prevalence of protozoan parasites detected from different water sources collected from Northern Parts of El-Minia Governorate.

City	Negative		Positive		Total	
	N	%	N	%	N	%
Maghagha	31	55.4	25	44.6	56	100
El-Edwa	36	64.3	20	35.7	56	100
Beni-Mazar	23	41.1	33	58.9	56	100
Matai	30	53.6	26	46.4	56	100
Samalut	32	57.2	24	42.8	56	100
El-Minia	44	78.6	12	21.4	56	100
Total number	196	58.3	140	41.7	336	100