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Detection and Enumeration of Waterborne Parasitic Protozoa from Different Water Sources in Three Different Governorates of Egypt

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ABSTRACT

Over the past few decades, there have been steady increases in the number of water-borne complaint outbreaks and the emergence of recently identified waterborne freeloaders. In the present work, a survey study was conducted in three different Egyptian governorates (Giza, Qalyubia, and Menoufia) during the period from March 2022 to January 2023 to identify and quantify water-borne freeloaders using different ways, including membrane filtration. A total of 430 water samples were collected from the study areas. *Giardia cysts* were detected in 8 samples, while *Cryptosporidium oocysts* were recorded in 28 samples. Additionally, excrescencies of *E. histolytica* were found in 8 samples, and *E. coli* excrescencies were found in 5 samples. About 26.3% of stream water samples and 21.4% of household water tanks were positive for water-borne parasites in Qalyubia Governorate, while 5.0% of stream water samples and 2.9% of household water tanks were positive for water-borne parasites in Giza Governorate. In Menoufia Governorate, about 15.0% of stream water samples and 13.3% of household water tanks were positive for water-borne parasites. The drinking water samples were negative for water-borne parasites in the three different governorates. The overall frequency of water-borne parasites in these three governorates (Giza, Qalyubia, and Menoufia) was 16.3%, 2.7% and 10.0%, respectively. In conclusion, this study could serve as a birth surveillance of waterborne freeloaders in different areas in Egypt. These results emphasized the significance of screening water sources to help prevent the potential spread of parasitic protozoan and other water-borne conditions, especially in areas that are near agricultural fields.

INTRODUCTION

Over the past few decades, there has been an increase in the number of water-borne complaint outbreaks and the emergence of recently honored waterborne spongers. Several factors that contribute to the spread of these conditions, include water, heavy rains, and agrarian remainders which transfer the spongers to the water face from the soil. The aim of this study was to assess the presence of waterborne parasitic protozoa in the swash and drinking water of Al-Wahdaa and Al-Rasheed Drinking Project, as well as in the river and drinking water tanks from some regions of Egypt. Waterborne conditions are linked to a significant complaint burden worldwide. About 1.8– 3.5 million imdividuals are affected by recreational water-borneails (RWI) in the USA alone. RWI is associated with water body contamination caused by contagious agents from sewer overflows **(Craig, 2010)**.

The epidemiology of protozoan waterborne conditions has changed. There was an increase in several waterborne outbreaks which is attributed, not only to increased vulnerability to conditions but also to the emergence of recently honored waterborne spongers **(Collins & Wright, 1997)**.

Cryptosporidium and *Giardia* are protozoan spongers known to be the most important cause of waterborne outbreaks worldwide **(Adam, 1991; O'Donoghue, 1995; Gad *et al*., 2020)**. *Cryptosporidium* spp. are intracellular, extra-cytoplasmic protozoan spongers with a monoxenous (single host) life cycle **(Ghazy *et al*., 2015)**. The members of the genus *Cryptosporidium* were first honored as mortal pathogens in 1976 **(White, 2010)**. They invade the microvillus border of the gastrointestinal epithelium in both humans and numerous wild and domestic animals **(De Graaf *et al*., 1999)**. *Cryptosporidium* belong to the phylum Apicomplexa **(O'Donoghue, 1995)**. They have lost the apicoplast organelle, as well as genomes for both the plastids and the mitochondria **(Ryan & Hijjawi, 2015)**. However, the genes associated with apical complex organelles (a group of organelles set up at the apical end of the organism) are present **(Sanderson *et al*., 2008)**.

Cryptosporidium spp. has numerous unique features that distinguish them from other protozoa, including (a) unusual position within the host cell, sequestered between the cell cytoplasm and cell membrane, (b) capability to initiate tone-infection, and (c) ingrain resistance to detergents **(Ghazy *et al*., 2015; Moussa *et al*., 2023)**. Because *Cryptosporidium spp.* oocysts are typically present in water samples in low concentrations and there are limited enrichment methods available, their detection and recovery rely on filtering or centrifuging the water sample, followed by meticulous examination for oocysts. Water samples are routinely tested using a standardized system USEPA Method 1623 which involves filtration (using membrane or cartridge pollutants), or nonstop inflow centrifugation of large volumes of water (minimum 10L) to concentrate suspended patches, followed by picky attention of *Cryptosporidium* oocysts and *Giardia* excrescencies using immunomagnetic separation (IMS), and staining with fluorescently labeled monoclonal antibodies, as well as 4 ′, 6-diamidino-2-phenylindole (DAPI), followed by luminescence microscopy. While Method 1623 is widely utilized, there are three primary approaches to analyzing water samples for *Cryptosporidium* and *Giardia*: careful examination, selective isolation of target organisms, and detection, identification, and confirmation. These methods can also be implemented using a range of essential techniques and technologies **(Efstratiou *et al*., 2017)**. For instance, flotation can replace the costly IMS method for detecting oocysts and cysts, while PCR (polymerase chain reaction)-based techniques enable discovery, facilitating the isolation of parasite species and genotype assemblages, an impossibility with microscopy alone. Various methods exist for the detection, identification, and investigation of *Cryptosporidium* spp. in both laboratory and field settings. These styles have their pros and cons, similar to discovery limit, perceptivity, particularity, cost, processing time, position of difficulty, and outfit demand; not every system would be applicable for routine quantification of *Cryptosporidium* spp. in water bodies.

Lautenschlager *et al*. (2013) bandied a fashion for the discovery and recitation of waterborne pathogens with delicacy using DNA uprooted from water samples, depending on the perceptivity and the utility of molecular styles similar to polymerase chain reaction (PCR) and hybridizations on DNA microarrays, and discarding methodological limitations and high costs of direct culturing. As a result, mortal conditioning agrarian, urban, and stormwater runoff, effluent from sewage treatment plants and industries, and leaching from septic systems can all contribute to the proliferation of blue-green algae.

The present study commenced by screening for ecological hazards in water quality, which involved field observations of specific physicochemical parameters and recording the transmission of waterborne parasitic protozoa. Additionally, we investigated natural contaminants such as parasitic oocysts and cysts, including *Cryptosporidium*, *Giardia*, and free-living amoebae.

MATERIALS AND METHODS

Water sample collection and parasite concentration

A survey study was conducted in three different governorates (Giza, Qalyubia, and Menoufia/ Egypt) during the period from March 2022 to January 2023. Water sample collection and parasite concentration were performed using membrane filtration **(EPA, 2001a)**. Parasitic excrescencies were separated from debris by Percoll- sucrose flotation **(Khoza, 2010)**.

Water filtration

Five- 10L of water samples were concentrated using membrane filtration **(EPA, 2001)**. A 25mm borderline, 1.0µm- severance- sizepolycarbonate (PCTE) membrane sludge (Whatman) was placed on a glass chimney stack mound filtration assembly (Millipore, Inc., Bedford, Mass.) and 20 of the sludge elute was filtered under vacuum (15lb/ in^2). For high cloudy water, multiple membrane pollutants were used for each sample before the final filtered sediments were combined. The deposit was washed with 10- 50mL eluting result (phosphate-softenedsaline (PBS), 1 Tween 80, and 1 sodium dodecyl sulfate. Parasitic excrescencies were further concentrated by centrifugation in 50mL falcon tubes at 2,500rpm for 20min in an S70D (MLW, Germany) centrifuge. The supernatant was aspirated just above the bullet, and the bullet was resuspended in the remaining supernatant. The sample concentrates were saved in an equal volume of 5 formalin and cooled until flotation on Percoll-sucrose slants.

Percoll-sucrose flotation fashion

Each water sample was mixed with 30mL Percoll-sucrose flotation effect with specific graveness of 1.18 in a 100ml beaker. Moreover, it was poured into a 200ml- volume cylinder vessel with a wide face area of 5cm borderline and left for 10min. The sample was covered with a plastic distance of equal size to the face area of the vessel and left for 20min. The plastic distance was precisely removed and irrigated into a new beaker. The same plastic distance was reused and the same process was repeated thrice. The total results after flushing plastic distance were combined and centrifuged at 3,500rpm for 10min, sluggishly accelerating the centrifuge over a 30-second interval up to the speed where the tubes are vertical to avoid dismembering the interface. Furthermore, at the end of centrifugation, sediment settled slowly, and the supernatant was removed. 2ml of distilled water was added and completely mixed with the bullet. A 25µl of each sample was examined under a light microscope at × 400 exaggerations. For enhancing oocyst identification, immunofluorescence assay (FA) fashion was performed on floated samples as well.

Microscopic examination of water samples

One drop of the deposit was placed on each slide, which was then stained using Ziehl-Neelsen/ Acid-Fast stain for the identification of *Cryptosporidium* as per **Henricksen & Pohlenz (1981)**, or stained with Lugol's iodine for the detection of parasitic oocysts and cysts, according to the method of **Garcia *et al.* (2003)**. Direct smears were examined under the light microscope using 10 and 40 objective lenses.

Detection of free-living amoeba

Each collected water sample was independently concentrated and filtered using cellulose nitrate membranes (0.45µm severance size and 47mm in borderline). The sludge was also transferred aseptically to the face of a 1.5 agar plate of non-nutrient agar (NNA) made with runner amoebae saline, covered by a thin estate of *Escherichia coli*, or sludge transferred to a parasite pad impregnated with a suitable picky medium and incubated. All dressed plates were incubated at 37 °C for up to 14 days with quotidian examination using an inverted light microscope for the presence of any amoebic growth. Bacterial (and other) cells trapped on the membrane grew into colonies that were counted, and bacterial viscosity was calculated. Colonies were allowed to develop on the face of the sludge and were directly counted and examined. All visible colonies were counted, and the results were reported as log colony forming units CFU mL −1.

RESULTS

Prevalence of water-borne protozoa contamination in different water sources

A total of 430 water samples were collected in the study areas. Two hundred and twenty water samples were from Qalyubia Governorate, 110 water samples were from Giza Governorate, and 100 water samples were from Menoufia Governorate. Table (1) shows the prevalence of water-borne protozoa in different water sources in the three studied areas. It was observed that in Qalyubia Governorate, water from the streams had the highest prevalence of 26.3 and 21.4% in household water tanks. Water samples from the streams in Giza Governorate recorded the lowest prevalence of 4.5 and 4.7% in streams and water tanks respectively. In Menoufia Governorate, water from the streams measured 15%, while the household water tanks contained 13.3%. No parasitic contamination (0%) was observed in any drinking water samples in the three studied areas.

Table 1. Prevalence of water-borne protozoa contamination in different water sources

Studied area	Number of samples	Number of positivity	Positivity %
Qalyubia Governorate	220	36/220	16.3 %
-Streams water	80/220	21	26.3 %
-Tanks water	70/220	15	21.4 %
-Drinking water	70/220	0	0 %
Giza Governorate	110	3/110	2.7 %
-Streams water	40/110	2	5 %
-Tanks water	35/110	1	2.9 %
-Drinking water	35/110	0	0 %
Menoufia Governorate	100	10/100	10 %
-Streams water	40/100	6	15 %
-Tanks water	30/100	4	13.3 %
-Drinking water	30/100	0	0 %

Parasitic protozoa of Qalyubia water samples

Table (2) shows that in Qalyubia Governorate, *Cryptosporidium* oocysts were detected in 13.8% of stream water samples and 12.9% of household water tanks. Moreover, *Giardia* cysts were detected in 5.0% of stream water samples and 4.3% of household water tanks. *E. histolytica* cysts were detected in 5% of stream water samples and 2.9% of household water tanks, while *E. coli* were detected in 2.5% of stream water samples and 1.4% of household water tank.

Table 2. Microscopic identification of water-borne protozoa in Qalyubia water samples

Water sample	Stream water	%	Tanks water	%	Drinking water	%
Giardia cysts	4/80	5.0 %	3/70	4.3 %	0/70	0 %
Cryptosporidium oocysts	11/80	13.8 %	9/70	12.9 %	0 /70	0 %
E. histolytica cysts	4/80	5.0 %	2/70	2.9 %	0 /70	0 %
E. coli cysts	2/80	2.5 %	1/70	1.4 %	0/70	0 %

Parasitic protozoa of Giza water samples

Microscopic identification of water-borne diseases in different water samples revealed thatin Giza Governorate, *Cryptosporidium oocysts* were found in 5% of stream water samples and 0% of household water tanks. Moreover, *Giardia* cysts were not detected in either stream water samples or household water tanks. Furthermore, *E. histolytica* cysts were absent in stream water samples and present in 2.9% of household water tanks, while *E. coli* was not detected in either stream water samples or household water tanks, as indicated in Table (3).

Table 3. Microscopic identification of water-borne Protozoa in Giza water samples

Water sample	Stream water	%	Tanks water	%	Drinking water	%
***Giardia* cysts**	0/40	0 %	0/35	0 %	0/35	0 %
Cryptosporidium oocysts	2/40	5 %	0/35	0 %	0 /35	0 %
E. histolytica cysts	0/40	0 %	1/35	2.9 %	0 /35	0 %
E. coli cysts	0/40	0 %	0/35	0 %	0/35	0 %

Parasitic protozoa of Menoufia water samples

Microscopic identification of water-borne diseases in different water samples revealed that in Menoufia Governorate, *Cryptosporidium* oocysts were detected in 10% of stream water samples and 6.7% of household water tanks. Moreover, *Giardia* cysts were detected in 2.5% of stream water samples and were absent in household water tanks. *E. histolytica* cysts were absent in stream water samples and 3.3 % of household water tanks, while E. coli was detected in 2.5% of stream water samples and 3.3% of household water tank, as indicated in Table (4).

Table 4. Microscopic identification of water-borne Protozoa in Menoufia water samples

Water sample	Stream water	%	Tanks water	%	Drinking water	%
***Giardia* cysts**	1/40	2.5 %	0/30	0 %	0/30	0 %
Cryptosporidium oocysts	4/40	10 %	2/30	6.7 %	0 /30	0 %
E. histolytica cysts	0/40	0 %	1/30	3.3 %	0 /30	0 %
E. coli cysts	1/40	2.5 %	1/30	3.3 %	0/30	0 %

DISCUSSION

Worldwide, it is estimated that 80 of wastewater is discharged into the terrain without sufficient treatment **(UN 2017)**. Additionally, at least 2 billion people use a drinking water source contaminated with feces **(WHO 2016)**. Waterborne diseases are estimated to be responsible for between 1.6 and 12 million deaths annually **(Roeger *et al*., 2018; Xagoraraki & O'Brien, 2020)**. Although the burden is the loftiest in developing countries, outbreaks of complaint still occur in developed countries and the global burden is estimated at 12 billion US bonesper time **(Alhamlan *et al*., 2015)**. Waterborne pathogens include bacteria (e.g. *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Vibrio cholerae*), contagions (e.g. Norovirus, Adenovirus, Poliovirus), protozoa (*Cryptosporidium* spp. and *Giardia* spp.) and helminths (e.g., *Ascaris* spp. and *Trichuris* spp.).

The present study screened for ecological pitfalls in water quality, including discovery, frequency, and webbing for the natural adulterants such as parasitic oocysts and excrescencies, *Cryptosporidium*, *Giardia*, and free-living amoebae. The study also assessed the frequency of water-borne complaint impurity in different water sources in the three studied areas. It was observed that in Qalyubia Governorate, water from the aqueducts had the loftiest frequency of 26.3 and 21.4 % in ménage water tanks. Water samples from the aqueducts in Giza Governorate recorded the smallest frequency of 4.5 and 4.7% in aqueducts and water tanks independently. In Menoufia Governorate, water from the aqueducts recorded 15%, and the menage water tanks recorded 13.3%. No parasitic impurity (0 %) was observed in any drinking water samples in the three studied areas. In line with these findings, **Gad *et al*. (2020)** conducted a study where they collected a total of 110 vegetable samples from agricultural fields across three regions in Giza Governorate, Egypt. Additionally, they collected 36 irrigation water samples (from both ground and surface freshwater) from the same agricultural fields. **Gad *et al.* (2020)** reported that the most prevalent type of enteric parasite found was microsporidia spores, with incidences of 18.2% in field vegetables, 18.3% in harvested vegetables, and 11.1% in irrigation water. Furthermore, *Cryptosporidium* oocysts were also detected in the irrigation water samples (11.1%).

In Qalyubia Governorate, *Cryptosporidium* oocysts were found in 13.8% of stream water samples and 12.9% of household water tanks. Additionally, *Giardia* cysts were found in 5.0% of stream water samples and 4.3% of household water tanks. *E. histolytica* excrescencies were set up in 5 sluice water samples and 2.9% menage water tanks, while *E. coli* was set up in 2.5% of sluice water samples and 1.4% of menage water tanks.

Another study conducted by **El-Tantawy *et al.* (2016)** investigated the surveillance of *Cryptosporidium* spp. oocysts, *Giardia* spp., and *Entamoeba* sp. cysts at a single time point. The study aimed to assess the correlation between the abundance of these pathogens and environmental factors in the vicinity of several water treatment facilities in Dakahlia Governorate.

Raw water samples were collected monthly from January 2014 to December 2014 from the bay of each installation. They found that the prevalence rates of different spongers were as follows; *Cryptosporidium* spp. (43.12), *Giardia* spp. (33.94), and *Entamoeba* spp. (22.93).

Al-Baytee *et al.* (2012) reported the prevalence of *Cryptosporidium* oocysts in water sources in Mansoria megacity, Diala Province, during the period from November 2008 to December 2009. They found that oocysts were present in tap water samples at a rate of 22%

only during the spring season. However, oocysts were detected in household water tanks at a rate of 32%. The frequency of oocysts decreased from 72% in spring to 16% in summer.

On the other hand, **Osman *et al.* (2010)** demonstrated in their study that free-living amoebae were present with a prevalence of 1.6 and 2.6% in the main water supplies of the megacities of Helwan and El-Giza, Egypt, respectively. Additionally, free-living amoebae are the vectors for some species of pathogenic bacteria to humans **(Abu Kwaik *et al*., 1998)**. The results agree with those of **CoGkun *et. al*. (2013)** concerning the frequency of free-living amoeba in valve water at 33 (22 %) of the total 150 samples for the fiefdom of Sivas, Turkey.

CONCLUSION

In conclusion, predicated on the results attained, this study could serve as a birth surveillance of waterborne diseases in different areas in Egypt. Future studies are recommended involving collecting large samples from other areas from Egypt and introducing further environmental toxicology, particularly heavy substances.

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