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Use of microscopic and molecular techniques to assess removal of parasitic protozoa via conventional and compact drinking water treatment processes

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ABSTRACT

The delivery of safe drinking water services depends on effective role played by stakeholders. From the parasitological point of view, the Egyptian standards for drinking water denied the presence of any type of living protozoan parasites in potable water produced for human use. In the present study, raw and treated water samples were separately collected from a conventional drinking water treatment plant (DWTP) and a compact unit. They were concentrated through nitrocellulose membrane filters (0.45μm pore size). The concentrate of each sample was examined with both light microscopy and PCR. Six genera of parasitic Protozoa (*Giardia, Cryptosporidium, Entamoeba, Balantidium, Cyclospora* and *Isospora*) and a group of microsporidial spores were detected in inlet water samples of the two examined treatment systems. Microscopically, the obtained data declared that 70.8% and 83.3% of inlet water from conventional DWTP and compact unit, respectively were contaminated with parasitic protozoa. The encountered parasitic protozoa in inlets of examined DWTPs were only microsporidial spores representing 8.3% and 16.7% of treated water from conventional DWTP and compact unit, respectively. Removal of protozoan parasites was higher in conventional DWTP (82.4%) than in compact unit (75%). Molecularly, the presence of protozoan parasites in the outlet of the compact unit was higher than in the outlet of the conventional DWTP. In general, molecular detection of protozoan parasites revealed a little bit lower or equal (in some instances) prevalence than that obtained by microscopy. In conclusion, most of inlet water samples from both conventional DWTP and compact unit were contaminated with protozoan parasites. Although conventional DWTP was more effective than compact unit for removing parasitic protozoa, l some of these parasites could be still detected in treated water and thus reach to consumers and cause health hazards.

INTRODUCTION

Protozoa include a diverse collection of parasites consisting of single cell; some of them are controversial for the water industry. Intestinal parasitic protozoa can easily get access to water supplies and thus caused plentiful water-related outbreaks. Moreover, these protozoan parasites are invulnerable to deactivation by chemical germicides applied in drinking water treatment. These parasites generally result in dysentery gastroenteritis of varying intensity, although more serious sequels

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(including death) can take place (APHA, 2018). Waterborne diseases caused by viruses, bacteria, protozoa and helminthes are the most current and widespread health hazard related to drinking water (WHO, 2011).

Successful removal of protozoan parasites in drinking water treatment processes depends mainly on early detection and identification of these organisms to prevent any spread or outbreak into a new region (Blackman *et al*., 2018). Microbiological contamination of treated water in distribution systems can occur. When protozoan parasites entered the distribution system, the consumers will be exposed to infection. Disinfectants may not be sufficient to get rid of the contamination (WHO, 2019).

In the United States, 18% of drinking water-related outbreaks between 1971 and 2006 were due to protozoa (Craun *et al*., 2010). Of the 325 water-related protozoan disease outbreaks announced worldwide, most were caused by *Cryptosporidium* spp. (51%) and *Giardia duodenalis* (41%) (Karanis *et al*., 2007). The rest were due to *Cyclospora cayetanensis* (1.8%), *Entamoeba histolytica* (2.8%), *Isospora belli* (0.9%), *Toxoplasma gondii* (0.9%), and *Blastocystis hominis* (0.6%). *Encephalitozoon* spp., *Enterocytozoon bieneusi, Balantidium coli*, *Acanthamoeba* spp., and *Naegleria fowleri* were encountered in 0.3% of outbreaks, but a conclusive association among drinking water and disease outbreaks has not been yet determined for some of these organisms. Many parasitic protozoa form dormant stages (cyst, oocyst, or spore) that allow them to resist drinking water treatment practices and survive in the environment. The main transmission route of these parasitic protozoa is the fecal-oral (Gill and Fast, 2006; APHA, 2018).

A conventional drinking water treatment plant contains four different stages (WHO, 2004), beginning from the inlet water (raw fresh water). Raw fresh water of the intake is pumped in pipes having rough metal sieves with 4cm pore size for prevention of indelicate matters from getting access with siphoned water. The sieved raw water is pumped to coagulation and precipitation ponds where it is mixed with aluminum sulfate to help in the flocculation and precipitation of the debris and microorganisms found in raw water. After that, the clear water on the top of sedimentation ponds is collected and passed on sand filters to get rid of the remaining microorganisms found in raw water. At the end, the clear water is collected in storage tanks where it is injected with chlorine dose of 2mg/liter for disinfection (WHO, 2004).

The disinfected water is pumped in the distribution systems to the consumers as a drinking water. The major two types of drinking water treatment plants are conventional drinking water treatment plant (DWTP) and compact unit (CU). Conventional water treatment plant produces a largest quantity of water than compact unit, so they were widely used in large municipal water systems by the 1920s. Rapid filters use relatively coarse sand and other granular media to remove impurities and grains that have been trapped in a floc through the use of chemicals—typically alum for flocculation. After that, the unfiltered water flows via the filter medium under pumped pressure and the flock material is trapped in the sand matrix. Compact drinking water treatment plant contains separate consequent containers (each one represents different treatment processes) for the production of drinking water to small communities. Typical systems include coagulation and flocculation lamella plates settling, sand filter and activated carbon filtration, polishing 5 or 10 micro cartridge

filters, followed by chlorination or UV. Standard systems can supply drinking water for populations from 100 to 25,000 people (https://www.rwlwater.com/compact-water-treatment-plants/).

The most commonly applied drinking water disinfectant is chlorine and its derivatives. Ozonation, ultraviolet irradiation, chloramination and application of chlorine dioxide are also used. These methods are very effecient in getting ride of bacteria and can be easily applied for inactivation of viruses (depending on type), and some may inactivate trophic stages of protozoa, including *Giardia* and *Cryptosporidium*. For effective removal or inactivation of protozoan cysts / oocysts, filtration with the aid of coagulation and flocculation (to reduce particles and turbidity) followed by disinfection (by one or a combination of disinfectants) is the most practical method (WHO, 2011).

It is substantial that an overall management strategy is accomplished in which various embracing, including protection of source water and adequate treatment stages, as well as protection during storage and distribution, are used in association with disinfection to remove or prevent microbiological contamination (WHO, 2011). So, the overall objective of this study is to evaluate the removal of parasitic protozoa via two different systems for drinking water treatment production.

MATERIALS AND METHODS

Samples and sampling sites

Water samples were collected from two different drinking water treatment processes (Imbaba conventional drinking water treatment plant and Geziret Elwarrak compact unit) in Giza district. Four different sampling sites namely:
- Inlet (surface or freshwater) of a conventional drinking water treatment plant.
- Outlet (finished or completely treated drinking water) of a conventional drinking water treatment plant.
- Inlet (surface or freshwater) of a compact unit.
- Outlet of (finished or completely treated drinking water) a compact unit.

Water samples were collected along one year period (from February 2017 to January 2018) for the detection of parasitic protozoa. Collected water samples were transported in ice-box at 4-8°C to the Environmental Parasitology Lab., National Research Centre, at the same day of collection (HPA, 2004; ISO/FDIS, 2006). Water samples (twenty litres from each sampling site) were separately collected in a clean plastic container and used for the detection of parasitic protozoa.

Concentration of enteric protozoan parasites

Each collected sample (20 liters volume) was filtered through 0.45µm Nitrocellulose membranes (142mm in diameter) by using stainless steel pressure filter holder (Sartorius). After filtration, the membrane was eluted with 200ml distilled water that were then centrifuged at 2000xg for 15min. Obtained sediments were divided into two equal parts; one for microscopic examination and the other was stored in -20°C for further molecular analysis.

Light microscopic detection of enteric protozoan parasites

Sediments of drinking water were directly examined microscopically then left to air drying and staining with acid fast trichrome, while sediments of surface water

were subjected to flotation using zinc sulphate (specific gravity 1.18) and upper 1ml of supernatant was collected and directly examined microscopically then left to air drying and stained with acid fast trichrome. Direct examination permitted detection of comparatively large protozoan cyst / oocyst such as *Giardia, Entamoeba, Balantidium* and *Isospora* (UK Standards for Microbiology Investigations Staining Procedures, 2015), while stained sediments facilitated the detection of smaller organisms like *Cryptosporidium* spp., *Cyclospora cayetanensis* and microsporidia (Ignatius *et al*., 1997).

Molecular detection of parasitic protozoa

Stored samples in -20°C for molecular analysis were subjected to DNA extraction using the QIAamp Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with the modifications described by Plutzer *et al.* (2010). Briefly, 1mL of the pellet obtained from a purified sample was mixed with 180µl of lysis buffer (buffer ATL), followed by 15 freeze-thaw cycles in liquid nitrogen (until the whole sample was completely frozen) and a water bath at 65°C (until the whole sample was completely thawed) to disrupt the (oo)cyst walls of protozoan parasites. Subsequently, the DNA extraction protocol recommended by the manufacturer was followed. The DNA extract was stored in the freezer at -20°C until needed for further testing. The PCR reactions were done using different primer pairs for *Entamoeba histolytica*, *Cryptosporidium* spp., *Giardia lamblia, Balantidium coli, Cyclospora cayetanensis, Isospora belli* and intestinal microsporidia (**Table 1**).

Table 1: Specific primers used for molecular detection of tested parasitic protozoa

Parasite	Primer	Primer sequence 5`-3`	Target genome	Product length (bp)	Reference
Entamoeba. histolytica	EntaF EhR	atgcacgagagcgaaagcat gatctagaaacaatgcttctct	18S rDNA gene	167	**Hamzah** ***et al*****., 2006**
Cryptosporidium spp	Cry-15 Cry-9	ggactgaaatacaggcattatcttg gtagataatggaagagattgtg	cowp gene	553	**Spano** ***et al*****., 1997**
Giardia lamblia	GlF GlR	aatctgttgacttaagggagta attgagtcattatagggattgt	Protease gene	463	**Bairami** ***et a*****l., 2016**
Balantidium coli	*B. coli*-F *B. coli*-R	aacctggttgatcctgccagt tgatccttctgcaggttcacctac	*Balantidium*r RNA gene	1543	**Nilles-Bije and Rivera, 2010**
Cyclospora cayetanensis	CCITS2-F CCITS2-R	gcagtcacaggaggcatatatcc atgagagacctcacagccaaac	rDNA gene	116	**Lalonde and Gajadhar, 2008**
Isospora belli	IsoFO IsoRO	gtgcctcttcctctggaagg gcactccacccagttaagtgc	rRNA gene	559	**Müller** ***et al*****., 2000**
Microsporidia	Pmp1 Pmp2 NA2	caccaggttgattctgcctgac cctctccggaaccaaaccctg agaaagagctatcaatctgt	rRNA gene	240–279	**Fedorko** ***et al*****., 1995**

Amplification of each protozoan DNA was performed using GoTaq G2 Green Master Mix (Promega, USA) according to the manufacturer manual. PCR reaction mixture per sample consisted of 12.5µl master mix, 3µl template DNA, 1µl of each primer (conc. 10pmol) and 7.5µl nuclease-free water. PCR thermal profiles for the detection of protozoan parasites were shown in **Table 2**. All PCR products were visualized as previously mentioned using 2% agarose gel stained with ethidium bromide.

Statistical analysis

The obtained data were analyzed by Paired t-test and two samples t-test using Minitab statistical program. *P*-value <0.05 was considered significant (Wild, 2005).

Table 2: PCR thermal profile for tested parasitic protozoa

Protozoa	Pre-denaturation	Cycles	Final extension	Reference
Entamoeba histolytica	95°C for 3min	**35** 95°C for 30sec 58°C for 30sec 72°C for 90sec	72°C for 10min	**EL-Sabbagh, 2010**
Cryptosporidium spp	95°C for 10min	**40** 95°C for 30 sec 57°C for 30sec 72°C for 30sec	72°C for 10min.	**EL-Sabbagh, 2010**
Giardia lamblia	95°C for 3min	**35** 94°C for 30sec 55°C for 30sec 72°C for 30sec	72°C for 10min	**Bairami *et al*., 2016**
Balantidium coli	95°C for 3min	**35** 95°C for 30sec 54°C for 90sec 72°C for 90sec	72°C for 10min	**Nilles-Bije and Rivera, 2010**
Cyclospora cayetanensis	95°C for 10min	**40** 95°C for 30sec 59°C for 30sec 72°C for 30sec	72°C for 10min.	**Lalonde and Gajadhar, 2008**
Isospora belli	95°C for 3min	**35** 94°C for 30sec 61°C for 30sec 72°C for 30sec	72°C for 10min	**Müller *et al*., 2000**
Microsporidia	95°C for 3min	**35** 94°C for 30sec 60°C for 30sec 72°C for 30sec	72°C for 10min	**Fedorko *et al*., 1995**

RESULTS AND DISCUSSION

The quality of source water should be routinely characterized to ensure the microbiological integrity of drinking water. Surveillance of source water for protozoan parasites can be targeted through the use of information about sources of fecal contamination, together with historical data on river flow rainfall and turbidity, to help identifying conditions that may possibly lead to peak events (Federal-Provincial-Territorial Committee on Health and the Environment, 2019).

Concerning parasitic protozoa, it was obvious that examination of 48 collected raw water samples revealed that parasitic protozoa were found in 77.1% (37 ⁄ 48) of raw inlet water samples examined by using light microcopy technique. The Morphologic identification of these detected parasitic protozoa revealed that they were belonging to six different genera (*Giardia, Cryptosporidium, Entamoeba, Balantidium, Cyclospora* and *Isospora*) and a group of microsporidia. The lowest occurrence of these parasitic protozoa (2.1%) was recorded by *Cyclospora* cysts; they were detected in only 1/48 examined raw water samples. Both *Balantidium* and *Isospora* were detected in 4.17% (2/48) of examined raw water samples. *Entamoeba*, *Giardia*, and *Cryptosporidium* were detected in 14.6% (7/48), 16.7% (8/48), and 29.2% (14/48) of examined raw water samples, respectively. The highest occurrence

(70.8%) was recorded by microsporidial spores; they were found in 34/48 examined raw water samples **(Figure 1)**.

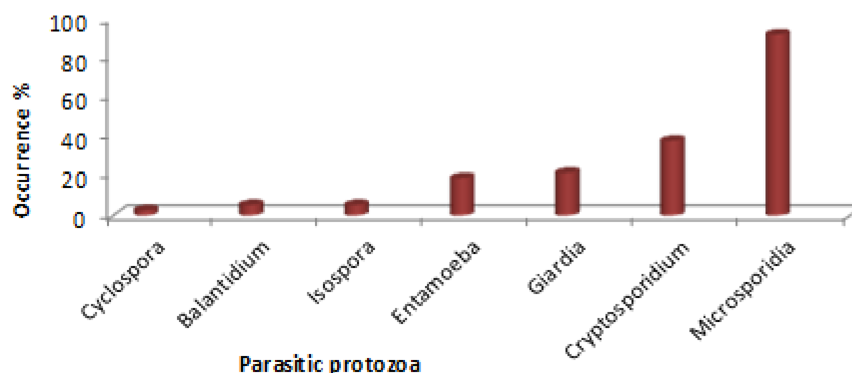

Figure 1: Occurrence of protozoan parasites in raw water

The multi-barrier approach (e.g. conventional DWTP and compact unit) is the best solution to reduce enteric protozoan parasites and other waterborne pathogens in drinking water. Many studies are dealing with a particular organism or a group of organisms through drinking water treatment steps, but a little are concerned with all of the enteric protozoan parasites as the present study declared. Intestinal parasites are parasites that can invade the gut of humans and other vertebrates. These parasites can stay throughout the host body, but most prefer the inner lining of intestinal tract. Means of exposure comprise ingestion and drinking of contaminated water (Patz *et al*., 2000; Renslo and McKerrow, 2006).

Parasitic protozoa, especially enteric ones, have been linked with drinking water related outbreaks. They may be present in water following direct or indirect pollution by the feces of humans or other vertebrates. Fecal-oral transmission is a common route of infection with *Cryptosporidium* and *Giardia*. Theoretically, a single cyst/oocyst of a protozoan parasite would be enough to provoke infection. Other investigations studies have declared that the dose required to provoke infection is usually more than a single cyst and is dependent on the virulence of the particular parasite strain (Federal-Provincial-Territorial Committee on Health and the Environment, 2019). According to world health organization (WHO) 80% of diseases are water borne. Drinking water in various countries does not meet WHO standards (Khan *et.al*., 2013). About 3.1% of deaths occur due to the unhygienic and poor quality of water (Pawari and Gawande, 2015).

The present work showed that the conventional DWTP system could remove 82.4% of protozoan parasites in raw water, while the compact unit could remove a lesser percentage (75%) of them **(Table 3)**.

Table 3: Removal of parasitic protozoa through drinking water treatment plants.

	Parasitic protozoa			
	Conventional DWTP		Compact DWTP	
	Raw	Finished	Raw	Finished
+ve samples	**17**	**3**	**20**	**5**
Removed		**14**		**15**
Removal %		**82.4**		**75.0**

Concerning parasitic protozoa, only microsporidial spores and *Cryptosporidium* oocysts were found in finished (completely treated drinking water) water samples **(Table 4)**.

Table 4: Occurrence protozoan parasites in raw and finished water samples collected from both conventional DWTP and compact unit.

	+ ve / examined (%)			
	Conventional DWTP		**Compact DWTP**	
	Raw	**Finished**	**Raw**	**Finished**
Entamoeba	3/24 (12.5)	0/24 (0.0)	4/24 (16.7)	0/24 (0.0)
Cryptosporidium	6/24 (25.0)	0/24 (0.0)	8/24 (33.3)	1/24 (4.2)
Giardia	4/24 (16.7)	0/24 (0.0)	4/24 (16.7)	0/24 (0.0)
Balantidium	0/24 (0.0)	0/24 (0.0)	2/24 (8.3)	0/24 (0.0)
Cyclospora	0/24 (0.0)	0/24 (0.0)	1/24 (4.2)	0/24 (0.0)
Isospora	1/24 (4.2)	0/24 (0.0)	1/24 (4.2)	0/24 (0.0)
Microsporidia	15/24 (62.5)	3/24 (12.5)	19/24 (79.2)	4/24 (16.7)

Statistically, the present work showed that conventional DWTP has a strong significant impact for removal of parasitic protozoa (P-value = 0.000) **(Table 5)**. Moreover, the compact drinking water treatment plant also had a major role for the removal of parasitic protozoa (P-value = 0.000) **(Table 6)**.

Table 5: Statistical analysis of the mean removal rates of protozoan parasites in conventional DWTP by using microscopic technique.

Water samples	**N**	**Mean**	**P - value**
Raw compact DWTP	**24**	**0.708333**	**0.000**
Finished compact DWTP	**24**	**0.125000**	

Table 6: Statistical analysis of the mean removal rates of protozoan parasites in compact unit by using microscopic technique.

Water samples	**N**	**Mean**	**P - value**
Raw compact unit	**24**	**0.833333**	**0.000**
Finished compact unit	**24**	**0.208333**	

In other studies, a comparison between efficiency of 2 DWTPs in Sharkia governorate declared that the removal of microsporidial spores from drinking water reached 100% and 96.8% in 2 different drinking water treatment facilities (Saad, 2016). In another study, *Cryptosporidium* oocysts and *Giardia* cysts, which were detected in raw inlet water of 2 drinking water treatment facilities, were completely removed from treated drinking water at the final step of treatment processes (Ali *et al*., 2004). The presence of protozoan parasites in raw water used as a water source for DWTPs represents a major risk of contamination.

Due to the possible health risk, comprehensive monitoring is recommended to be followed on recreational lakes, and other sources of water (Onichandran *et al*., 2013). Cysts and oocysts are truly electronegative (Capizzi-Banas *et al*., 2004), a property utilized at both the coagulation and filtration steps. At the coagulation step, precipitate catchment using metal hydroxides can decrease cyst and oocyst concentration by more than 99%. Filtration through diatomaceous earth can be highly effective, while the use of electropositive coatings, such as hydrous iron aluminum oxide, promotes granular filtration effectiveness several folds (Betancourt and Rose, 2004).

The present results revealed that most prevalent organisms in raw water were microsporidial spores which were detected in 62.5% and 79.2% of inlet water samples

from conventional and compact DWTPs, respectively. Also, microsporidial spores were the only organism detected in finished water of the 2 treatment systems (**Table 4**).

In a recent study conducted in Greater Cairo, Egypt by Al-Herrawy *et al.* (2017), it was clear that intestinal microsporidia prevailed in 8.8% of citizens and the presence of *Enterocytozoon bieneusi* was higher than *Encephalitozoon intestinalis* in the tested human stool samples. Stool of those infected persons, in addition to feces of infected animals and birds (Gad, 2010; Al-Herrawy and Gad, 2016), might be indirectly a source of contamination of surface Nile water which is the main supply of drinking water in that province.

Cryptosporidium parvum and *G. lamblia* are the most prevalent waterborne parasites causing diarrhea in developing countries and till now little attention by authorities in relieving their effects has been recieved. The presence of such protozoan parasites in the water sources can cause a high risk to the citizens (States *et al*., 1997). In Egypt, water used by citizens inhabiting Nile delta regions comes from Nile River and its channels where most domestic animals inhabit these areas. All the citizens living at the boundary of Nile River and its tributaries in rural areas have their houses situated near to the water bodies from which they bring water for domestic uses.

The presence of such protozoan parasitic stages in completely treated drinking water produced for human consumption was considered a health risk hazards, especially for those with immune-deficiency, children and elder ones (Dowd *et al*., 2003). Parasitic protozoa, such as coccidia (*Cryptosporidium parvum, Cyclospora* sp., and *Isospora belli*), intestinal microsporidia (emerging parasites that infect the gastrointestinal tract (Gomes *et al*., 2002), and *Giardia lamblia* which causes diarrhea mainly in children (States *et al*., 1997) can be spread via water. In developing countries, over one billion persons do not have access to clean, properly treated water, and approximately three billion people do not have proximity to adequate sanitary facilities (Kraszewski, 2001). This lack of clean water was probably a consequence of outgrowing environmental disintegration, reduction of water resources, and increasing contamination of water bodied with domestic wastewater and industrial discharges, which cause microorganisms (from soil, feces, decomposing organic matter and other pollutant sources) to spread into water (Pedro and Germano, 2001).

Molecular identification of protozoan parasites in the present work revealed the existence of *Cryptosporidium* spp., *Giardia lamblia, Entamoeba histolytica* and microsporidia in the raw and finished water samples collected from both the conventional and compact DWTPs. *Isospora beli*, *Cyclospora cateytanensis* and *Balantidium coli* were not detected (**Table 7**). It is important to discern the different species of the *Entamoeba* complex since the only species clearly related with pathogenicity is *E. histolytica* and neither microscopy nor culture methods can discriminate between these *Entamoeba* species, the use of molecular techniques is a necessity (Fotedar *et al*., 2007).

Previous studies in Malaysia documented the detection of protozoan parasites, principally *Giardia* and *Cryptosporidium*, in drinking water treatment facilities. The overall occurrence of *Cryptosporidium* oocysts (18.8%) was shown to be lower compared to *Giardia* cysts (32.9%) (Ahmed *et al*., 1997; Tan, 1997). On the contrary in the present study, contaminated water samples with *Cryptosporidium* were greater

than those contaminated with *Giardia.* This difference might be due to difference in load of cysts and oocysts in feces and wastes that would contaminate surface water (Richard *et al*., 2016).

Table 7: Molecular occurrence of protozoan parasites in collected water samples

Parasitic protozoa	positive / examined (%)			
	Conventional DWTP		Compact unit	
	Raw	Finished	Raw	Finished
Entamoeba histolytica	3/24 (12.5)	0/24 (0.0)	3/24 (12.5)	0/24 (0.0)
Cryptosporidium spp.	4/24 (16.7)	0/24 (0.0)	4/24 (16.7)	0/24 (0.0)
Giardia lamblia	3/24 (12.5)	0/24 (0.0)	4/24 (16.7)	0/24 (0.0)
Balantidium coli	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)
Cyclospora caeytanensis	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)
Isospora beli	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)	0/24 (0.0)
Microsporidia	13/24 (54.2)	1/24 (4.2)	15/24 (62.5)	1/24 (4.2)

Parasitic protozoa such as *Balantidium coli*, *Isospora* and *Cyclospora caeytanensis* were not detected by PCR, although they were detected microscopically in low percentages **(Figure 2)**.

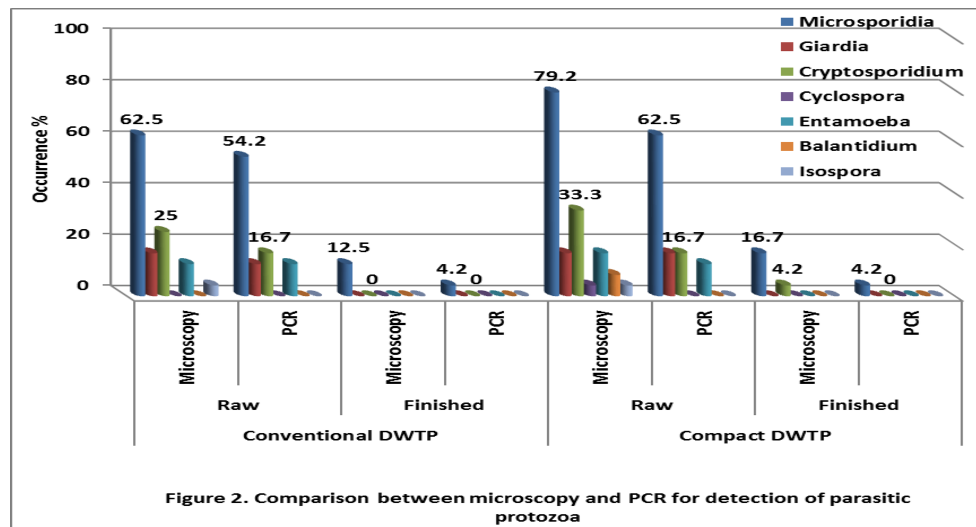

Figure 2. Comparison between microscopy and PCR for detection of parasitic protozoa

Other researchers denoted that still, traditional parasitological diagnosis based on the detection of enteric parasites by light microscopy will remain essential in the future, particularly in epidemiological surveys, but it depends on the parasitological experience of the laboratory staff (Parčina *et al*., 2018). Statistically, there was no significant difference (P-Value = 0.550) between using microscope and PCR for recognition of protozoan parasites in the examined water samples **(Table 8)**.

Table 8: Statistical analysis of the mean occurrence of protozoan parasites in water samples using two different methods (Microscope versus PCR)

Method	N.	Mean	P - value
Parasitic protozoa by microscopy	**24**	**0.708**	**0.550**
Parasitic protozoa by PCR	**24**	**0.625**	

Although dependent on technologically advanced equipment and still expensive, PCR assays may be of particular interest in endemic areas because repeatedly or chronically infected individuals living in these areas may shed parasites at considerably lower numbers than acutely infected travelers, which may lead to

decreased sensitivity of light microscopy and assays based on antigen detection (Ignatius *et al*., 2014). A major problem with the evaluation of detection assays, however, may arise when the target pathogens are absent or present at a very low prevalence in the study samples, which consequently hampers the ascertainment of the sensitivity of the assay (van Lieshout and Roestenberg, 2015).

CONCLUSION

The intakes of the examined drinking water treatment facilities were contaminated with intestinal parasitic protozoa that are considered a potential health threat. Regular examination of drinking water for the presence of protozoan parasites must be performed as a routine test to prevent incidence of waterborne parasitic outbreaks. Better monitoring and administration methods are needed to overcome the risk of waterborne transmission of pathogens, and efficient wastewater treatment is crucial to protect the public health against epidemic enteric parasitic infections. Statistically, the examined two drinking water treatment facilities had a significant role in removing the parasitic protozoa from the contaminated surface water.

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CONFLICT OF INTEREST

The authors announce that there is no conflict of interests.

REFERENCES

Ahmad, R.A.; Lee, E.; Tan, I.T.L. and Mohamad-Kamel, A.G. (1997). Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in raw and treated water from two water treatment plants in Selangor, Malaysia. Wat. Res., 31: 3132–3136.

Al-Herrawy, A.Z. and Gad, M.A. (2016). Microsporidial spores in fecal samples of some domesticated animals living in Giza, Egypt. Iran. J. Parasitol., 11(2): 195-203.

Al-Herrawy, A.Z.; Marouf, M.A. and Gad, M.A. (2017). *Acanthamoeba* species in tap water, Egypt. Int. J. Pharma. Clin. Res., 19: 21-25.

Ali, M.A.; Al-Herrawy, A.Z. and El-Hawaary, S.E. (2004). Detection of enteric viruses, *Giardia* and *Cryptosporidium* in two different types of drinking water treatment facilities. Water Res., 38:3931-3939.

APHA (2018). Standard method for the examination of water and wastewater. APHA, WEF and AWWA, DC 2011, Washington.

Bairami, A.; Rezaei, S. and Rezaeian, M. (2016). Evaluation of a new primer in comparison with microscopy for the detection of *Giardia lamblia* infection in stool samples. Iran. J. Parasitol., 11: 19–23.

Betancourt, W.Q. and Rose, J.B. (2004). Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. Vet. Parasitol., 9: 219-234.

Blackman, R.C.; Hänfling, B. and Lawson-Handley, L. (2018). The use of environmental DNA as an early warning tool in the detection of new freshwater invasive non-native species. CAB Reviews, 13: 1-15.

Capizzi-Banas, S.; Deloge, M.; Remy, M. and Schwartzbrod, J. (2004). Liming as an advanced treatment for sludge sanitisation: helminth eggs elimination - *Ascaris* eggs as model. Water Res., 38 (14-15): 3251-3258.

Craun, G.F.; Runkard, J.M.B.; Oder, J.S.Y.; Oberts, V.A.R.; Arpenter, J.C.; Ade, T.W.; Alderon, R.L.C.; Oberts, J.M.R.; Beach, M.J. and Roy, S.L. (2010). Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. Clin. Microbiol. Rev., 23(3): 507-528.

Dowd, S.E.; John, D.; Eliopolus, J.; Gerba, C.P.; Naranjo, J.; Klein, R.; Lopez, B.; de Mejia, M.; Mendoza, C.E. and Pepper, I.L. (2003). Confirmed detection of *Cyclospora cayetanesis, Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. J. Wat. Health, 1: 117-123.

El-Sabbagh, Y.A.H.H. (2010). Development of uniplex and multiplex PCR assays for the detection of human enteric protozoan pathogens. Ph.D. Thesis, University of Leicester.

Federal-Provincial-Territorial Committee on Health and the Environment (2019). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document - Enteric Protozoa: *Giardia* and *Cryptosporidium*. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Fedorko, D.P.; Nelson, N.A. and Cartwright, C.P. (1995). Identification of microsporidia in stool specimens by using PCR and restriction endonucleases. J. Clin. Microbiol., 33: 1739–1741.

Fotedar, R.; Stark, D.; Beebe, N.; Marriott, D.; Ellis, J. and Harkness, J. (2007). PCR detection of *Entamoeba histolytica, Entamoeba dispar* and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. J. Clin. Microbiol., 45: 1035–1037.

Gad, M.A. (2010). Prevalence of human intestinal microsporidiosis in Greater Cairo. M.Sc. Thesis, Faculty of Science, Ain Shams University.

Gill, E.E. and Fast, N.M. (2006). Assessing the microsporidia fungi relationship: Combined phylogenetic analysis of eight genes. Gene 375: 103-109.

Gomes, A.H.S.; Pacheco, M.A.S.R.; Fonseca, Y.S.K.; Cesar, N.P.A.; Dias, H.G.G. and Silva, R.P. (2002). Pesquisa de *Cryptosporidium* sp em águas de fontes naturais e comparação com análises bacteriológicas. [Research on *Crytosporidium* sp in water from natural springs and comparison with bacteriological analysis]. Rev. Inst. Adolfo. Lutz., 61(1): 59-63.

Hamzah, Z.; Petmitr, S.; Mungthin, M.; Leelayoova, S. and Chavalitshewinkoon-Petmitr, P. (2006). Differential detection of *Entamoeba histolytica, Entamoeba dispar,* and *Entamoeba moshkovskii* by a single-round PCR assay. J. Clin. Microbiol., 44: 3196–3200.

HPA (2004). Isolation and identification of *Acanthamoeba* species. W 17: Issue 2.

https://www.rwlwater.com/compactwater-treatment-plants.

Ignatius, R.; Gahutu, J.B.; Klotz, C.; Musemakweri, A.; Aebischer, T. and Mockenhaupt, F.P. (2014). Detection of *Giardia duodenalis* assemblage A and B isolates by immuno-chromatography in stool samples from Rwandan children. Clin. Microbiol. Infect., 20(10): O783–O785.

Ignatius, R.; Lehmann, M.; Miksits, K.; Regnath, T.; Arvand, M.; Engelmann, E.; Futh, U.; Hahn, H. and Wagner, J. (1997). A new acid-fast trichrome stain for simultaneous detection of *Cryptosporidium parvum* and microsporidial species in stool specimens. J. Clin. Microbiol., 35: 446-449.

ISO/FDIS 19458 (2006). Water quality — Sampling for microbiological analysis.

Karanis, P.; Kourent, C. and Smith, I.H. (2007). Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. J. Wat. Health., 5(1):1-38.

Khan, N.; Hussain, S.T.; Saboor, A.; Jamila, N. and Kim, K.S. (2013). Physiochemical investigation of the drinking water sources from Mardan, Khyber Pakhtunkhwa, Pakistan. Int. J. phys. Sci., 8(33):1661-1671.

Kraszewski, J. (2001). Water for people supports small systems for impoverished people worldwide. J. Am. Wat. Work. Assoc., 93: 36-37.

Lalonde, L.F. and Gajadhar, A.A. (2008). Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. App. Environ. Microbiol., 74: 4354–4358.

Müller, A.; Bialek, R.; Fätkenheuer, G.; Salzberger, B.; Diehl, V. and Franzen, C. (2000). Detection of *Isospora belli* by polymerase chain reaction using primers based on small-subunit ribosomal RNA sequences. Europ. J. Clin. Microbiol. Infect. Dis., 19: 631–634.

Nilles-Bije, M.L. and Rivera, W.L. (2010). Ultrastructural and molecular characterization of *Balantidium coli* isolated in the Philippines. Parasitol. Res., 106: 387-394.

Onichandran, S.; Kumar, T.; Lim, Y.A.; Sawangjaroen, N.; Andiappan, H.; Salibay, C.C.; Chye, T.T.; Ithoi, I.; Dungca, J.Z.; Sulaiman, W.Y.; Ling, L.Y. and Nissapatorn, V. (2013). Waterborne parasites and physico-chemical assessment of selected lakes in Malaysia. Parasitol. Res., 112: 4185–4191.

Parčina, M.; Reiter-Owona, I.; Mockenhaupt, F.P.; Vojvoda, V.; Gahutu, J.B.; Hoerauf, A. and Ignatius, R. (2018). Highly sensitive and specific detection of *Giardia duodenalis, Entamoeba histolytica*, and *Cryptosporidium* spp. in human stool samples by the BD MAX™ Enteric Parasite Panel. Parasitol. Res., 117:447–451.

Patz, J.A.; Grakzyk, T.K.; Geller, N. and Vittor, A.Y. (2000). Effects of environmental change on emerging parasitic diseases. Int. J. Parasitol., 30 (12):1395-1405.

Pawari, M.J. and Gawande S. (2013). Ground water pollution and its consequence. Int. J. Engin. Res. Gen. Sci., 3(4):773-76.

Pedro, M.L.G. and Germano, M.I.S. (2001). A água: um problema de seguran çanacional. [Water: A national security problem]. Rev. Hig. Aliment., 15: 15-18.

Plutzer, J.; Torokne, A. and Karanis, P. (2010). Combination of ARAD microfibre filtration and LAMP methodology for simple, rapid and cost-effective detection of human pathogenic *Giardia duodenalis* and *Cryptosporidium* spp. in drinking water. Lett. Appl. Microbiol., 50(1): 82-88.

Renslo, A.R. and McKerrow, J.H. (2006). Drug discovery and development for neglected parasitic diseases. Nat. Chem. Biol., 2 (12): 701-710.

Richard, R.L.; Ithoi, I.; Abd Majid, M.A.; Sulaiman, W.Y.; Tan, T.C.; Nissapatorn, V. and Lim, Y.A. (2016). Monitoring of waterborne parasites in two drinking water treatment plants: A Study in Sarawak, Malaysia. Int. J. Environ. Res. Pub. Health., 13 (7): 641.

Saad, A. (2016). Detection of microsporidia in water resources in Sharkeya governorate. M.Sc. Thesis, Faculty of Science, Zagazig University.

Spano, F.; Putignani, L.; McLauchlin, J.; Casemore, D.P. and Crisanti, A. (1997). PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum*, and between *C. parvum* isolates of human and animal origin. FEMS Microbiol. Lett., 150: 209–217.

States, S.; Stadterman, K.; Ammon, L.; Vogel, P.; Baldizar, J.; Wright, D.; Conley, L. and Sykora, J. (1997). Protozoa in river water: Sources, occurrence, and treatment. J. Am. Wat. Work. Assoc., 89: 74-83.

Tan, I.T.L. (1997). Pengesanan Protozoa Pathogen di TigaBuah Loji Pembersihan Air di Negeri Sembilan. M.Sc. Thesis, University of Kebangsaan, Malaysia.

UK Standards for Microbiology Investigations Staining Procedures (2015). Staining Procedures. Issue no: 2.1

Van Lieshout, L. and Roestenberg, M. (2015). Clinical consequences of new diagnostic tools for intestinal parasites. Clin. Microbiol. Infect., 21(6): 520–528.

WHO (2004). Water Treatment and Pathogen Control. IWA Publishing. Pp 1-107.

WHO (2011). Guidelines for drinking-water quality, fourth edition.

WHO (2019). Strengthening drinking-water surveillance using risk-based approaches. Copenhagen: WHO Regional Office for Europe. Licence: CC BY-NC-SA 3.0 IGO.

Wild D.J. (2005). MINITAB Release 14. J. Chem. Inf. Model. doi: 10.1021/ci040130h.

www.who.int/water_sanitation_health/publications/2011/dwq_chapters/en.