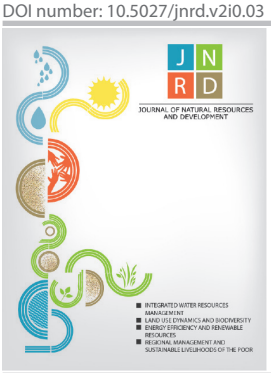

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Monitoring for the presence of parasitic protozoa and free-living amoebae in drinking water plants

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Abstract

Contamination of drinking water by microorganisms represents a major human health hazard in many parts of the world. The main objective of drinking water treatment is to provide microbiologically safe drinking water. The conventional drinking water treatment and disinfection has proved to be one of the major public health advances in modern times. A number of processes; namely water treatment, disinfection and changes influence the quality of drinking water delivered to the customer's tap during transport of treated water via the distribution system. At least 325 water-associated outbreaks of parasitic protozoan disease have reported. In this study, drinking water from treatment plants evaluated for the presence of parasitic protozoa. Water samples collected from two main points: (a) outlet of the water treatment plants (b) distribution system at different distances from the water treatment plants. Protozoa were concentrated from each water sample by adsorption and accumulation on the nitrocellulose membrane filters (0.45 µm pore size) and detected by conventional staining methods.

Introduction

Waterborne diseases occur worldwide. Outbreaks caused by the contamination of community water systems have the potential to cause diseases in large numbers of consumers. Waterborne outbreaks have economic consequences. Beyond the cost of health care for affected patients, their families and contacts, and the economic costs of illness and disease, they also create a lack of confidence in potable water quality and in the water industry in general. Interest in the contamination of drinking water by enteric pathogenic protozoa has increased considerably during the past three decades and the waterborne route (Panagiotis, *et. al.,* 2007) transmits a number of protozoan parasitic infections of humans.

Free-living amoebae (FLA) are the most prevalent protozoa found in the environment. FLA are isolated from soil, air, and water, dust, sewage, and sediments (Rodriguez-Zaragoza, 1994). They can colonize water systems and have been isolated from drinking water plants (Hoffmann and Michel, 2001; Thomas, *et. al.,* 2008), hospital water networks (Thomas, *et. al.,* 2006), domestic water networks (Kilvington, *et. al.,* 2004), and cooling towers. Among FLA, *Acanthamoeba species* are the most frequently found in human infections (Céline, *et. al.,* 2010). Pathogenic FLA, such as *Naegleria fowleri, Acanthamoeba spp., Balamuthia mandrillaris* and *Sappinia diploidea* can cause life-threatening infections in humans and animals (Schuster and Visvesvara, 2004; Daft, *et. al.,* 2005; Jonas Behets, *et. al.,* 2007). FLAs are also a factor for keratitis and encephalitis (Fields, *et. al.,* 2002, Akın, 2003: Dilara and Zuhal, 2011). They are responsible for human infections and can host pathogenic microorganisms.

Giardia lamblia and *Cryptosporidium parvum* are parasitic, intestinal protozoan responsible for disease outbreaks in humans. When

ingested in contaminated water, they cause giardiasis (beaver fever) and cryptosporidiosis. Symptoms include diarrhea, abdominal cramps, nausea, vomiting, chills, fever, dehydration, headaches, and malaise. Both parasites produce cysts that withstand harsh environmental conditions, lying dormant until ingestion. The levels of chlorine normally used to disinfect drinking water do not kill cysts. Both organisms reproduce in humans, domestic pets, livestock, and wildlife. Then are shed in fecal matter and spread via contaminated water (Anon, 1996; Barbara, 1997). Its occurrence is dependent on factors that include season, age and other demographic characteristics of a population; among children aged 1–5 years with diarrhea, *C. parvum* may be the most frequently found pathogen. Therefore, the three genera of waterborne protozoan pathogens are transmitted via the fecal–oral route and are important causes of waterborne outbreaks of gastroenteritis (Thurston-Enriquez, *et. al*., 2002), (Table 1).

Since the protozoa are typically related to faecal contamination of surface water, several studies have investigated the use of indicator bacteria to predict high levels of protozoa. However, no consistent relationship has been observed between indicator bacteria (thermotolerant coliform) levels and concentrations of *Giardia* or *Cryptosporidium*. Since (oo) cysts are much more persistent than coliforms and enterococci in water, it is likely that these bacteria are not valid indicators, especially if the contamination source is distant. Persistence of bacterial indicators (spores of *Clostridium perfringens*) may prove to be useful indicators for these protozoa (Hijnen, 1997). In the absence of valid surrogates, watershed assessment to determine local sources of contamination and define the amount of treatment necessary should include monitoring for protozoa, due to the fact that even in very low numbers, it poses a high risk to the consumer (Hibler and Hancock, 1990; Rose, 1990; Ali, *et. al.,* 2004; WHO, 2008). Protozoan parasitic cysts and oocysts are more resistant to certain water purification processes than bacterial indicators. Disinfection with chlorine has always been an important option for preventing transmission of waterborne pathogens. However, high resistance to chlorine disinfection, especially of *Cryptosporidium* oocysts (Whitmore, 1994), makes the process ineffective for oocyst inactivation in drinking water (WHO, 2008).

Table 1. Some parasitic protozoa and waterborne route of transmission

Organism	Disease / symptoms	Geographic distribution	Transmissive stage	Size (mm)	Infection route
Entamoeba histolytica	Dysentery, liver abscess	Cosmopolitan	Cyst	9 - 14.5	ingestion
Giardia duodenalis	Diarrhea, bad absorption	Cosmopolitan	Cyst	8 - 12	ingestion
Cryptosporidium spp.	Diarrhea	Cosmopolitan	Locust	4 - 6	ingestion
Balantidium coli	Diarrhea, dysentery	Cosmopolitan	Cyst	50 - 60	ingestion
Sarcocystis sp.	Diarrhea, muscle weakness	Cosmopolitan	Oocyst	7.5 - 17	ingestion
Toxoplasma gondii	Lymphadenopathy, fever, congenital infections	Cosmopolitan	Oocyst	10 - 12	ingestion
Cyclospora sp.	Protracted diarrhea	Cosmopolitan	Oocyst	8 - 10	ingestion
Microsporidia	Enteritis, hepatitis, peritonitis, keratoconjuntivitis	Cosmopolitan	Spore	1.8 - 5	ingestion/ contact with eyes

Source: Modified from Smith & Lloyd (1997)

Material and Methods

The methodology for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in water is completely different from the traditionally used for quantification of faecal indicator bacteria in the water industry. The procedure consists of three stages: (i) sample collection and concentration, (ii) separation of (oo) cysts from contaminating debris, and (iii) detection of (oo) cysts.

In this research, water samples were collected from water treatment plants drawing raw water from the Nile River during spring 2010. The monitoring study was carried out in El Monofya Governorate including three cities, Qwisna, Birket El Sabaa and Shibeen El Koom. From each city, two water treatment stations were evaluated for its water quality in outlet and from the distribution system (DS). Water samples of 20 L were collected from each station. Sodium thiosulfate (BDH Chemicals Ltd Poole England) was added to the chlorinated samples in a final concentration of 5 mg/L, to inactivate chlorine. Samples analysis looked for the presence of the protozoan parasites *Giardia, Cryptosporidium,* and *Amoeba*.

Physicochemical Analysis of Water

The following parameters were measured for all water samples: pH, turbidity, total suspended solids and residual chlorine concentration according to the Standard Methods (APHA, 2005).

Protozoa concentration

Giardia and *Cryptosporidium*

In each water sample, protozoa were collected from the nitrocellulose membrane according to the method of Payment, *et. al.* and 1989; Kfir, *et. al.* 1995. The pH of each sample was adjusted to 3.5. Every sample was filtered separately through a nitrocellulose membrane (0.45µm pore size, 142 mm diameter, Millipore).

The protozoan parasites *Giardia* and *Cryptosporidium,* that might be present on the surface of the membrane filter after sample filtration, were collected by soaking and thorough washing of the membrane in 20 mL of 5% formal saline [5% formaldehyde (Merck–Schuchardt) in 0.85% Na Cl (Sisco Res. lab. India)]. This washing solution was centrifuged (Hermel Z 323 K, Germany) at 4000 g for 6 minutes at room temperature and the produced pellet was re-suspended in 1mL of distilled water. A volume of 500 µl was used for microscopic examination.

Amoeba

Nonnutrient agar (NNA) (1.5 %) plates were used for the isolation of free-living amoeba (FLA) from water samples. Before the inoculation of the samples, NNA plates were coated with a dense suspension of heat inactivated *Escherichia coli*, which were prepared in Page Saline. The samples were filtered through a 0.45µm pore size cellulose nitrate membrane filter in vacuo. The filters were inverted on heat-inactivated *E. coli* treated 1.5% NNA plates. After the inoculation of the samples, all plates were incubated at 28°C and examined daily for 10 days using a light microscope (100x) to detect the presence of FLA (Schuster, 2002; Health Protection Agency 2004; Jeong and Yu, 2005; Ertabaklar, *et. al.,* 2007; Zuhal Zeybek, *et. al.,* 2010).

Microscopic Examination

Stained smears from formalin-fixed pellets of concentrated water samples were prepared and examined microscopically. Chlorazol black E (Sigma) was used for detection of *Giardia* cysts. For *Cryptosporidium* oocysts the modified Kinyoun acid-fast method was used, proposed by Alles and Coworkers (1995).

Results and Discussion

Waterborne diseases constitute a major human health problem worldwide. Many countries are concerned with the results of some studies of water distribution systems (DS). These studies were based on water quality evaluation in simulated model systems to assess the effects of disinfectants on pathogens in drinking water (Norton, *et. al.,* 2004; Williams, *et. al.,* 2004; Chauret, *et. al.,* 2005; Donlan, *et. al.,* 2005; Loret, *et. al.,* 2005; Van der Kooj, *et. al.,* 2005).

The results of physic-chemical analysis including pH, turbidity, TDS and residual chlorine of the six stations are shown in Table (2).

Table 2. Results of physicochemical parameters

Cities	Stations	pH	Turbidity	TDS	Residual chlorine	Sampling point
Qwisna City	Arab El Raml Station	7.6	2	666	1	Outlet
		7.5	3.2	652	0.1	Distribution system
	Main Quisna Station	7.6	1	435	0.2	Outlet
		7.7	1.6	439	0.8	Distribution system
Breket El Sabaa City	Meet faris Station	7.9	1.3	255	1.9	Outlet
		7.8	2.3	258	0.5	Distribution system
	El Roodaa Station	7.7	1.4	371	0.5	Outlet
		7.6	1.1	371	0.6	Distribution system
Shibeen El Koom City	Shibeen El Koom Station	8.1	0.8	252	1	Outlet
		8.0	0.9	250	0.8	Distribution system
	Meet Mousa station	7.9	0.1	256	1	Outlet
		7.9	0.3	259	0.8	Distribution system
Regulation N° 45, year 2007 for drinking water		6.5-8.5	1	1000	-	

FLA recorded negative results in the outlet of all the stations and also in the DS of all the stations. It is known that water temperature, pH, and free chlorine amounts affect FLA reproduction (Francine Marciano-Cabral, *et. al.,* 2010). As these amoebae are known to thrive at higher temperatures, their numbers might be higher in the DS following a warm summer season. In contrast, their numbers might be low in the spring following the colder winter temperatures. Hence, this was particularly agreed based in the results of spring sampling at detection levels, Table (3).

FLA that belong to the genus *Acanthamoeba* are widespread in the environment, including water. They are responsible for human infections and can host pathogenic microorganisms. Under unfavorable conditions, they form cysts with high levels of resistance to disinfection methods, thus potentially representing a threat to public health (Céline Coulon, *et. al.,* 2010). Due to their capacity to resist chemical and physical treatments used for drinking water production and distribution (Loret, *et. al.,* 2008; Thomas*et, al.,* 2008) they can colonize virtually any artificial water system.

Giardia and *Cryptosporidium* are protozoan parasites transmitted by contamination of the environment with resistant cysts and oocysts excreted by infected hosts (Marshall, *et. al.,* 1997). *Giardia lamblia* is the most commonly isolated intestinal protozoan parasite throughout the world and it is especially prevalent in children in developing countries (Bryan, *et. al.,* 1994). *Giardia* cysts have incriminated as causative agents of 19 and 36 waterborne protozoan outbreaks associated with recreational water and drinking water, respectively (Levy, *et. al.,* 1998). In Egypt, *Giardia lamblia* was detected in freshwater (Khairy, *et. al.,* 1987); finished water (Bassiouni, *et. al.,* 1988) and tap water (Abd El-Rahman, 1993).

In this study, the parasitic protozoa (*Giardia* and *Cryptosporidium*) results were different and variable between the six stations. In Qwisna city, two stations were evaluated: Arab El Raml Station and Main Qwisna Station. The parasitic parasites were recorded with positive result (+) in distribution system of Arab El Raml station, while the Outlet of Arab El Raml Station, Outlet of Main Qwisna Station and Distribution System of Main Qwisna Station gave negative (-) results. In Birket El Sabaa city the evaluated stations were Meet faris Station and El Roodaa Station. In the outlet of Meet faris Station, Distribution System of Meet faris Station and Distribution System of El Roodaa Station the results were positive (+). Meanwhile, the sample from Outlet of El Roodaa Station gave negative (-) results. Shibeen El Koom City was the third city including two stations, Shibeen El Koom Station and Meet Mousa Station. Although the Outlet of Shibeen El Koom Station gave negative results for parasitic protozoa, the distribution system of Shibeen El Koom Station had the highest positive presence (+++) of parasitic parasites. In addition, the outlet of Meet Mousa station and the distribution System of Meet Mousa Station gave also positive (+) results (Table 3).

Concerning cryptosporidiosis, different species of *Cryptosporidium* occur in different host groups but they cannot be distinguished simply based on host occurrence or parasite morphology. Infection with *Cryptosporidium* has been shown to be readily transmissible between hosts belonging to the same vertebrate classes' mammal-to mammal and bird-to-bird (Fayer, *et. al.,* 1997). *Cryptosporidium* oocysts were detected from different water types in many countries including Egypt (Marshall, *et. al.,* 1997; Xiao, *et. al.,* 2001; Ali, *et. al.,* 2004).

Table 3. Results of parasitic protozoa

Cities	Stations	Parasitic parasites	Freshwater living amoeba	Sampling point
Qwisna City	Arab El Raml Station	--	--	Outlet
		+	--	Distribution system
	Main Quisna Station	--	--	Outlet
		--	--	Distribution system
Breket El Sabaa City	Meet faris Station	+	--	Outlet
		+	--	Distribution system
	El Roodaa Station	--	--	Outlet
		+	--	Distribution system
Shibeen El Koom City	Shibeen El Koom Station	--	--	Outlet
		+++	--	Distribution system
	Meet Mousa station	+	--	Outlet
		+	--	Distribution system

(--) = 0
(+) = 1-10 organism/ mL
(++) = 11- 20 organism/ mL
(+++) = > 20 organism/ mL

The principal barrier for protozoa is physical removal by filtration. *Cryptosporidium* oocysts are relatively small, making them more difficult to remove than *Giardia* cysts. The higher removal rates were achieved when coagulant dose was applied to the water before filtration. Slow sand filtration efficiently removes (oo)cysts, but its efficiency is reduced at lower temperatures. Since sand filters employed in the treatment plant would not remove the diversity of small protists inhabiting the river, it is assumed that most protozoa are susceptible to the effects of chlorine at levels used, although there is very little comparable data available on this topic. A recent European study, reported that sand filters were colonized and may occasionally release FLA into filtered water (Thomas *et. al.,* 2008; Wendy, 2010).

Disinfection with chlorine has always been an important option for preventing transmission of waterborne pathogens. However high resistance to chlorine disinfection, especially of *Cryptosporidium* oocysts, makes the process ineffective for oocyst inactivation in drinking water.

Chlorine dioxide is slightly more effective, but still requires a high CT value (concentration (residual) of disinfectant C × contact time T) of 78 mg·min/litre for 90% inactivation of oocysts. *Giardia* is less resistant to chlorine: 99.99% reduction can be achieved with a CT of 180–530 mg·min/litre, depending on the temperature and pH of the water. At CT values of 4.7–28 mg·min/litre chlorine dioxide reduces Giardia by 99%. Disinfection with ozone is generally very expensive, but it is the most potent agent against (oo) cysts (WHO, 2004), (Table 4).

Table 4. Waterborne pathogens and their significance in water supplies (WHO 2004)

Pathogen	Health significance	Persistence in water supplies	Resistance to chlorine	Relative infectivity	Important animal source
Acanthamoeba spp.	High	Long	High	High	No
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply	High	High	No
Toxoplasma gondii	High	Long	High	High	Yes

Water distribution system makes water available to the consumers in proper quantity and pressure. Tap water should not contain microorganisms, parasites or substances that might represent a potential hazard for human health and it must meet the minimal requirements stipulated in regulation concerning the quality parameters of potable water (microbiological and chemical indicators). The quality of water delivered to the customers depends on (i) its initial chemical and physical composition, (ii) the proper choice of purification technology, (iii) technical conditions of water storage tanks and pipe network as well as (iv) hydraulic condition and exploitation manner of the water distribution system. Thus, water distribution system acts as large-scale chemical and biological reactors and sometimes, due to improper design or operation, can greatly modify the quality of water (e.g. long retention times which lead to water aging, reduced disinfectant residual and formation of disinfection sub-products, bacterial growth, appearance of taste and odor and so on).

Although studies of water DS have been performed in several countries, many of these have been based on the evaluation of the water quality in simulated model systems. Those models usually assess the effects of disinfectants on pathogens in drinking water (Norton, *et. al.,* 2004; Williams, *et. al.,* 2004; Chauret, *et. al.,* 2005; Donlan, *et. al.,* 2005; Loret, *et. al.,* 2005; Van der Kooj, *et. al.,* 2005). Microorganisms can enter the DS via cross-connections between drinking water and sewer lines, backflows, breakthroughs in drinking water, wastewater treatment plant operations, and leaking pipes, valves, joints and seals as well as contamination of the tap by the final users.

Conclusion

Contamination of the Nile River with faecal materials like pathogenic protozoa still represents an environmental health hazard in Egypt, especially in rural areas. Accordingly, prevention of the Nile River contamination will enhance the efficiency of drinking water treatment facilities for pathogens removal.

Prevention of the transmission of protozoan parasites through drinking water requires a multiple barrier approach: (i) protection of watersheds used for drinking water production against contamination with protozoa, (ii) adequate treatment of water—and (iii) verification by monitoring of water quality and operational parameters of the treatment effectiveness. Many water utilities use chlorine residual to inactivate potential pathogenic organisms and preserve water quality during distribution. Thus, controlling the residual chlorine concentration in drinking water is a very important aspect, since the decrease of chlorine (concentration below the minimal level) may cause secondary development of microorganisms and excessive chlorine concentration may cause formation of dangerous disinfection by-products. Disinfectant dose, contact time, residual disinfectant concentration at the end of the contact time, pH, and

temperature are commonly used to monitor the performance of disinfection processes. The most critical conditions for disinfection processes are low temperatures and high turbidity in the water to be treated.

Finally, the positive results in outlets samples may be due to failure of sand filters stage to remove pathogenic organisms, or the chlorine concentration was below the minimal level. In case of positive results in distribution system, this can be attributed to leaking pipes, valves, joints and seals, as well as contamination of the tap by the final users.

Recomendations

One of the most important aspects of watershed protection is the recognition of local sources of contamination with parasitic protozoa and the control of that contamination by diversion or treatment of discharges and reduction of direct input of faeces, especially in otherwise pristine waters, by people, farm animals, and wildlife or from manure storage. In addition, emphases on the application of quality control standards in drinking water purification plants, and periodic follow-up for its quality.

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