Detection rates of waterborne protozoa in water sources from Fayoum Governorate

Original Article

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ABSTRACT

Background: Waterborne protozoal infections are common health problems in many parts of the world especially in developing countries. Water is a major vehicle for transmission of protozoa such as *G. lamblia, Cryptosporidium* spp. as well as pathogenic and opportunistic free living amoeba (FLA).

Objective: This study aims to detect the presence of protozoal agents in tap water and storage water tanks at Fayoum Governorate, Egypt.

Material and Methods: A total of ninety five water samples were collected from different water sources, taps (65) and tanks (30), from 6 Fayoum districts. The samples were processed to detect the presence of *G. lamblia* cysts by Lugol's iodine stain, *Cryptosporidium* oocysts by modified Ziehl-Neelsen stain and FLA by cultivation. After cultivation, *Acanthamoeba* spp. were identified according to their morphological features and flagellation test was performed to detect amoeboflagellates.

Results: All water samples collected from tanks (100%) were contaminated by protozoa of medical importance, while only 6 (9.2%) of the tap water samples were pathogen free. The majority of water samples were contaminated with mixed protozoal infections. The overall detection rates of contaminants in water sources were 86.3%, 52.6%, 13.7% by FLA, *Cryptosporidium* spp. and *G. lamblia*, respectively.

Conclusions: The recorded detection rates of waterborne protozoa present a hazard to the community resulting in silent morbidities and mortalities. It is strongly recommended to adopt proper water safety measures.

INTRODUCTION

From the public health point of view, a dependable supply of safe drinking water is vital for daily life. Unfortunately, the same water that promotes life can also be the bearer of dangerous contaminants in the form of protozoa that contaminate drinking water supplies in developed and developing countries. These protozoa are *Giardia lamblia, Cryptosporidium* spp., pathogenic and opportunistic free living amoeba (FLA).

Cryptosporidium spp. and *G. lamblia* are major waterborne pathogens[1], transmitted as a result of water contamination with animal and human feces[2]. Cryptosporidiosis is a major cause of acute diarrhea in children and chronic persistent diarrhea in HIV infected individuals with low CD4 counts[3]. *G. lamblia* causes large numbers of gastrointestinal infections worldwide with complications that include steatorrhea, malabsorption and growth retardation in children[4,5]. Pathogenic and opportunistic FLA are aerobic eukaryotic protists that can potentially cause infections in humans and animals[6]. In addition, *Acanthamoeba* spp. is known to produce chronic granulomatous amoebic encephalitis[7] as well as corneal keratitis[8].

Diligent determination of dispersed waterborne protozoa in water sources is of fundamental importance, especially sources exposed to pollutants such as sewage, industrial discharge and human activities that are rarely subjected to minimal treatment before discharge into the Nile River. Possible reasons for constant contamination include inefficient coagulation, filtration and poor disinfection (e.g. no free-residual disinfectant and short contact times) during water treatment. Pathogenic microorganisms that evade treatment may survive and enter the piped distribution system and be the source of an important level of endemic disease in the population[9,10]. Therefore, the selection of suitable processes of treatment to remove this contamination is very important for safe water supply.

The safety of water is threatened by the use of old metal water distribution pipes which are more prone to develop

biofilms on their walls, nearness of the water distribution system to the sewage system and the use of water pumps to raise pressure of water in many houses induces vacuum that draws sewage water from broken water pipe system. These factors increase the percentage of protozoa in drinking water[11]. In many countries including Egypt, the populations consume water from water storage roof tanks. The water quality changes according to the storage time, condition and cleaning of those tanks. Tanks may be contaminated from a variety of sources including piping, tank construction materials, sealed tank covers or breathing outlets improperly designed, insects, animal and bird feces that might gain access to the tanks, which leads to increase in the microbial contamination[12].

The present study aims at detecting the presence of medically important protozoa in tap and water tanks at Fayoum Governorate, Egypt.

MATERIALS AND METHODS

This descriptive analytical study was conducted during the period from May, 2015 to October, 2015 as a part of a M.Sc. fulfillment. The samples were processed at the Central Microbiology/Parasitology laboratory of Fayoum drinking water and sanitation company.

Study area: Water samples were collected from Fayoum governorate. This governorate is bound from the east, west and north by Giza governorate, while its southern boundary is Beni Suef governorate. Samples were collected from 6 districts at Fayoum governorate (Fayoum, Sennuris, Tamia, Yousif El-Sedek, Ibshawai, Itsa) (Figure 1); and both water tap and water tanks were collected.

Sample collection, transportation and storage: Ninety five water samples were collected in 1 L and 2 L sterile polypropylene containers. The 1 L samples were used for detection of free living amoeba and the 2 L samples were used for screening of *Cryptosporidium*
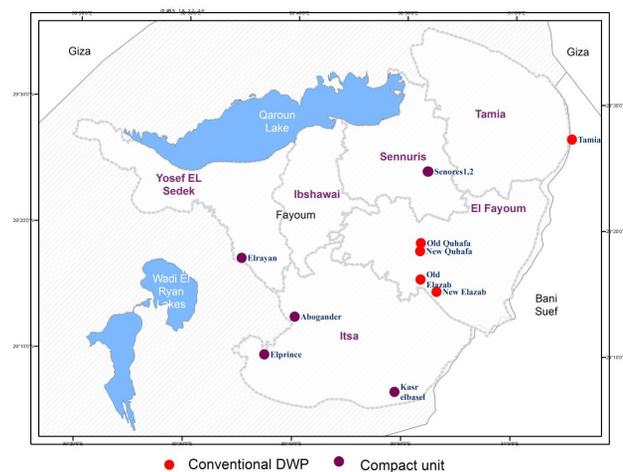

Fig. 1: Detailed main water treatment plants (WTPs) map for the studied area.
DWP: Dwelling water pump.

spp. and *G. lamblia*. The samples were collected from households, schools and mosques. Samples were transmitted immediately using an ice box for sample preservation, to the Central Microbiology/Parasitology Laboratory of Fayoum drinking water and sanitation company, for analysis. The samples were refrigerated at 4°C until processed within 24 h.

Laboratory methods

Lugol's iodine stain technique for detection of *G. lamblia* cyst: Lugol's iodine stains the nuclear structures of *Giardia* cysts maximizing recovery of cysts. Each water sample was concentrated by centrifugation at 5000 × g for 20 min. The supernatant was poured off leaving the sediment which was stained with Lugol's iodine [13]. Direct smears were examined under the light microscope using 10 and 40 objective lenses to identify *G. lamblia* cysts

Modified Ziehl-Neelsen (MZN) staining for detection of *Cryptosporidium* oocysts: To maximize recovery of oocysts, the samples were centrifuged at 5000 × g for 20 min to concentrate the oocysts. MZN technique stains oocysts red against a blue background. Samples were screened by 40 X and confirmed by 100 X objectives [14].

Cultivation and identification of FLA: Collected water samples were separately concentrated using the membrane filtration technique. One liter of each water sample was filtered through a nitro-cellulose membrane filter (0.45 μm pore size and 47 mm in diameter) that was then removed before complete dryness. Each membrane was inverted on the surface of a non-nutrient agar plate lined with *Escherichia coli* and incubated at 35°C [15]. The plates were observed daily for 14 days using an inverted microscope to detect sluggishly moving trophozoites with hyaline pseudopodium protrusions, or double walled cysts with polygonal shaped interior or parallel inner and outer walls. Flagellation test was carried out using distilled water to detect amoeboflagellates (FLA other than *Acanthamoeba* spp.) [16]. Identification was based on the morphological characteristics of trophic, temporary flagellate and cyst stages [17,18].

Ethical consideration: Approval to conduct this study was obtained from Fayoum drinking water and sanitation company. Permission of households and authorities in schools and mosques was obtained for collection of water samples, after explaining the aim of the study.

RESULTS

The overall detection rates in both water sources (Table 1) collected from 6 districts in Fayoum governorate were 72.7%, 53.7%, 52.6%, 13.7% for *Acanthamoeba* spp.,

FLA other than *Acanthamoeba* spp., *Cryptosporidium* spp. and *G. lamblia*, respectively (Figure 2).

All water samples were contaminated with medically important protozoa except for seven tap water samples that were pathogen free (Table 2). Most tap and tank water samples proved to be contaminated with more than one parasite; 42/65 samples (64.6%) and 23/30 samples (76.7%), respectively. The most common single contamination was with *Acanthamoeba* spp. detected in 8 tap water and 5 tank samples. The most common associations in double contamination were with *Acanthamoeba* spp. and amoeboflagellates (10 tap water and 6 tank samples) and *Cryptosporidium* spp. (8 tap water and 3 tank samples). The most common association in triple infection was between *Acanthamoeba* spp., *Cryptosporidium* spp. and amoeboflagellates which were detected in 11 tap water and 9 tank samples. Only one tank water sample was found to be contaminated by all the mentioned protozoa (Table 2). The distribution of FLA in water tanks varied. *Acanthamoeba* spp. alone contaminated 40% of the collected samples, amoeboflagellates were found in 6.7% and both types of FLA were found in 53.3%. From tap water, *Acanthamoeba* spp. alone contaminated 29.2% of samples, while amoeboflagellates were found in 12.3% and both FLA were found in 38.5% (Figure 3).

Table 1: Detection rates of waterborne protozoa in water sources from 6 districts of Fayoum Governorate.

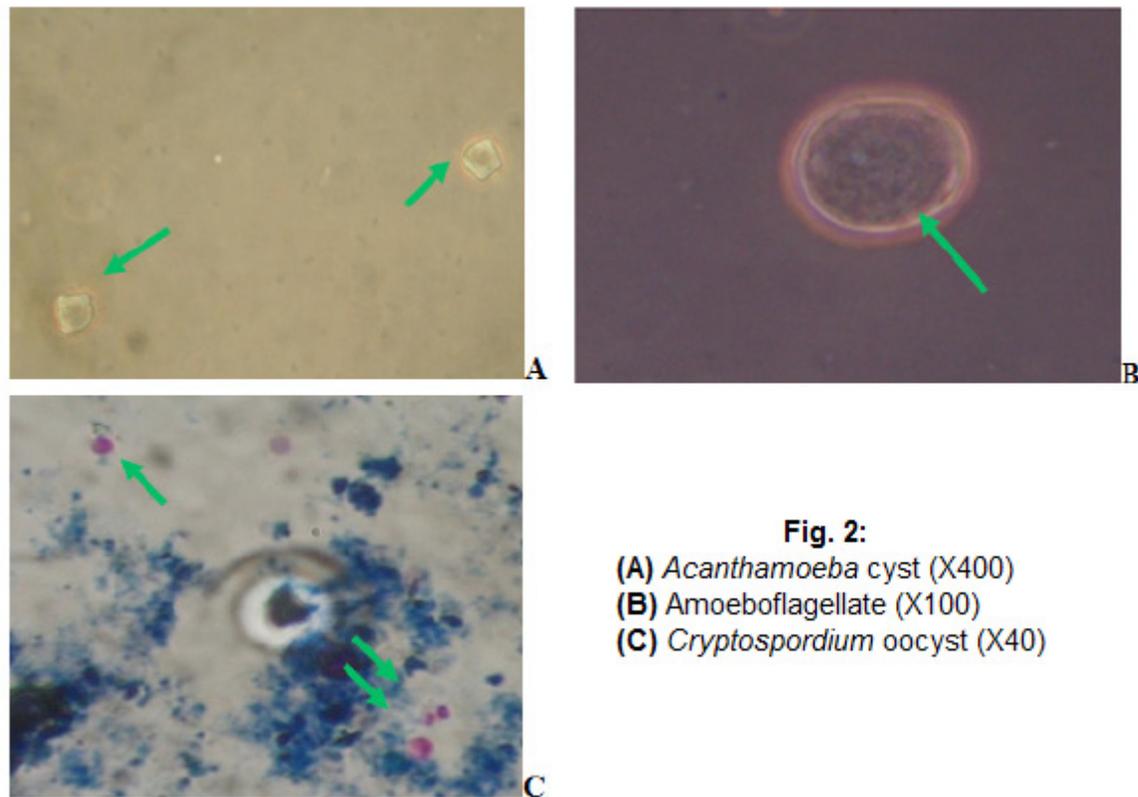|	Tap		Tank		Total	
	No.	%	No.	%	No.	%
Free living amoeba (FLA)	52	80	30	100	82	86.3
Acanthamoeba **spp.**	44	67.7	28	93.3	72	72.7
Amoeboflagellates	33	50.8	18	60	51	53.7
Cryptosporidium **spp.**	34	52.3	16	53.3	50	52.6
G. lamblia	8	12.3	5	16.7	13	13.7

Fig. 2:
(A) *Acanthamoeba* cyst (X400)
(B) Amoeboflagellate (X100)
(C) *Cryptospordium* oocyst (X40)

Table 2: Distribution of contaminated water sources from 6 districts of Fayoum Governorate.

|	Tap		Tank	
	No.	%	No.	%
Total number with single contamination	16	24.6	7	23.3
Total number with mixed contamination	42	64.6	23	76.7
Pathogen free	7	10.8	0	0
Total	65	100	30	100
Single				
Acanthamoeba spp.	8	50.0	5	71.4
Amoeboflagellates	2	12.5	2	28.6
G. lamblia	0	0	0	0
Cryptosporidium spp.	6	37.5	0	0
Total	16	100	7	100
Mixed				
Double				
Acanthamoeba – Cryptosporidium	8	19	3	13
Acanthamoeba - G. lamblia	0	0	1	4.4
Acanthamoeba – Amoeboflagellates	10	23.8	6	26.1
Amoeboflagellates – *Cryptosporidium*	5	11.9	0	0
Triple				
Acanthamoeba – Cryptosporidium - G. lamblia	3	7.0	3	13
Acanthamoeba – Cryptosporidium - Amoeboflagellates	11	26.2	9	39.1
Acanthamoeba - G. lamblia - Amoeboflagellates	4	9.5	0	0
Amoeboflagellates - *Cryptosporidium - G. lamblia*	1	2.4	0	0
Quadruple: All organisms	0	0	1	4.4
Total	42	100	23	100
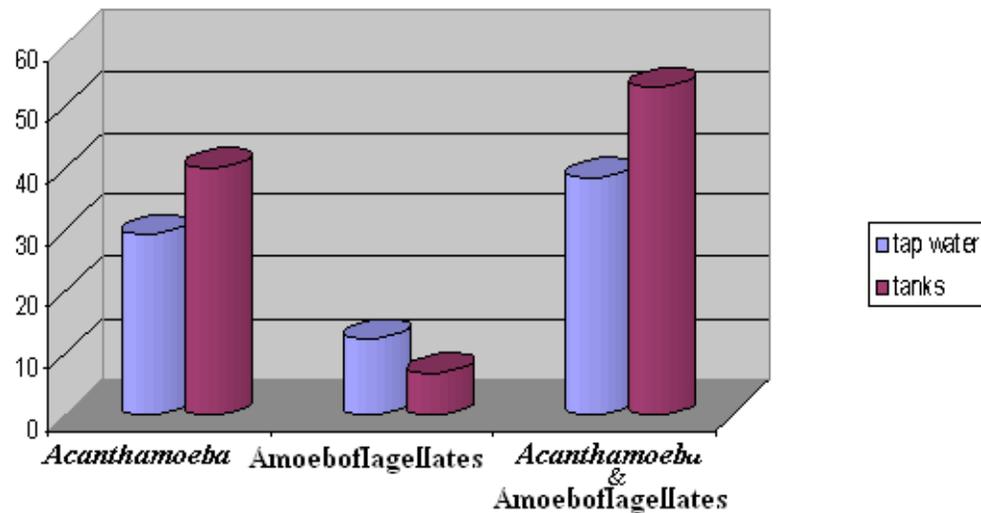
Fig. 3. Distribution of free living amoeba in drinking water samples collected from tap water and water tanks, respectively.

DISCUSSION

One of the most important problems that the majority of the world's population is facing is the shortage of safe water for consumption. This problem is a challenge in poor developing countries as it causes serious health problems. In addition, contamination of drinking water sources with protozoan pathogens threatens millions of people in developed countries and results in severe morbidities and mortalities[2]. The problem is particularly evident in rural Egyptian villages, where the main source of water is the Nile river that is affected by many polluting activities such as sewage, industrial discharge as well as human activities. In some areas of Egypt, sewage and industrial discharge may be subjected to minimal treatment and discharged directly into the Nile river and its lakes, and canals. Higher levels of pathogens in surface water supplies, including density and variety, are expected in areas where treatment of sewage and industrial discharges is marginal or nonexistent[19]. Meanwhile, adequate and fully operational conventional drinking water treatment processes (coagulation, sedimentation, filtration, and disinfection) share in the reduction of microbial contaminants of public health concern in raw source water[20]. Effective coagulation and filtration process in water plants are important components of the conventional treatment process that control the passage of waterborne protozoan cysts/oocysts to the distribution system[21]. Therefore, determination of how much our water sources are contaminated with water-borne protozoa is of prime importance.

In the present work, 95 drinking water samples collected from Fayoum governorate were examined for potential protozoal contamination. The most frequent parasitic contamination was FLA with 86.3% overall detection rate, and 80% and 100% contamination of tap water and water tanks. Several studies were conducted in Egypt and recorded FLA in tap water with lower occurrence rates; 4% in samples collected from different Egyptian governorates[22], 41.7% in Gharbeya governorate[23] and 60.4% in Giza governorate[24]. On the other hand, similar result (88.9%) was obtained in Qalyubia governorate[23]. In other countries, a low detection rate (9.3%) was recoded in USA[25], whereas variable rates were reported in several studies all over the world; 46.9% in Korea[26], 23% in Nicaragua[27], 22.79% in Brazil[28], 29.4% and 22% in Turkey[29,30]. However, similar results were obtained on examination of household tap water samples in UK (89%)[31] and in Ohio, USA (79%)[25]. Conflicting results on FLAs contamination in tap water from different countries might be attributed to factors known to influence their presence, such as water source, water treatment method, geographic location, and differences in water temperature[25]. High contamination of tap water with FLAs might lead to more biofilm formation along the piped water network. This is consistent with findings from a study that showed settlement of microorganisms on the inner surfaces of water pipes that would later become a source of secondary microbial contamination[32]. Therefore, high contamination of the piped water network with FLAs would definitely result in nosocomial infections due to the survival and persistence of these pathogens in the biofilms inside the storage tanks of hospitals. It was reported that FLAs are known to thrive in areas containing high bacterial content that provides them with nutrition[7], a conclusion that is consistent with our results in detection of FLAs in all samples collected from water tanks. It was also reported that most FLAs are known to facilitate intracellular multiplication of *Legionella pneumophila, Vibrio cholerae, Bacillus anthracis* and *Mycobacterium tuberculosis* which are responsible for legionellosis, cholera, anthrax and tuberculosis, respectively[7]. On the other hand, different FLA genera infect human *via* sniffing of contaminated water, and they are transmitted to man causing diseases through washing before praying (ablution).

It is well known that FLA is a broad term encompassing a variety of agents including both pathogenic and non-pathogenic organisms, and the common pathogenic agents include *Acanthamoeba, Naegleria, Balamuthia* and *Sappinia*. In the present study, it was shown that the detected FLAs belonged to either *Acanthamoeba* spp. or other unknown amoeboflagellates based on the microscopic morphological features and flagellation test. Our results showed that *Acanthamoeba* spp. were the most prevalent FLA as detected in 93.3% and 67.7% of water tanks and tap drinking water samples. Other workers in Egypt recorded *Acanthamoeba* spp. in 41.7% and 88.9% in tap water in Gharbeya and Qalyubia governorates, respectively[23]. Lower rates were recorded in a Nicaraguan study (21%, 19% and 29%) of all their water sources, tap water and water tanks, respectively[27]. Furthermore, low detection rates (26.8%, 5.3% and 9.1%) were recorded in tap water in United Kingdom, Turkey and Philippines, respectively[30,31,33]. It was reported that *Acanthamoeba* spp. are more resistant to harsh conditions and can survive for a long time[34,35]. However, the variability of detection rates in several reports is due to variable seasonal temperature which affects the intensity and variety of *Acanthamoeba* spp.[36]. Our high detection rate was attributed to the time of study as the samples were collected from May to October, 2015, and most of *Acanthamoeba* spp. prevail during summer months[37]. Another explanation for the high detection rate of *Acanthamoeba* spp. is that our water samples may be rich in total dissolved solids which provide nutrition and support growth of *Acanthamoeba* spp.[38,39]. Beside its medical importance for causing chronic granulomatous amoebic encephalitis and corneal keratitis, *Acanthamoeba* spp. can harbor echo-virus[6], and mimivirus which were discovered in *A. polyphaga*[40].

Amoeboflagellates were found in 53.7% in all water sources and in 50.8% and 60% of tap water and water tank samples, respectively. The lower detection rates of amoeboflagellates in comparison to *Acanthamoeba* spp. in the present study may be due to their domestic distribution

instead of different habitat conditions (air, water, soil) for the latter[37,41]. Low detection rates were recorded in Nicaragua (9%, 4% and 5%), respectively[27].

The detection rate of *Cryptosporidium* oocysts in all water sources was 52.6 %, 52.3 % in tap water, and 53.3% in water tanks. For tap water samples, lower detection rates were recorded in Egypt; 3.1% in Dakhahlia governorate[42], and 13% in Gharbeya governorate[43]. However, the detection rate reaches 25% in Brazil[44], 34.6% in Iran[45] and 41.6% in Baghdad[46]. Similar results were obtained in El-Minia governorate where the investigators collected 10 ml of water from different water sources (canals, tanks, and tap water) and they detected *Cryptosporidium* oocysts in 53% of their water samples[19]. High detection rate (92%) was recorded on examining drinking water sources in Argentina and the investigators found *Cryptosporidium* oocysts in a concentration ranging from 20-539 oocysts/100 liter[47]. By PCR, *Cryptosporidium* oocysts were detected in all examined water sources (100%) in UK[48], in 3.1% of tap water samples in Ismailia[49], in 10.2% and 40.1% in samples collected from treated drinking water in Portugal[50] and Spain[51], respectively. In the Spanish study, the investigators used immunofluorescent assay (IFA), and a similar result was obtained (40.9%). As regards water tanks, *Cryptosporidium* oocysts were detected in 3% in Dakahlia governorate[52]. In Jordan, the investigators reported detection of *Cryptosporidium* oocysts in water sediment of home storage tanks of patients with diarrhea[53]. In Seoul, Republic of Korea, a waterborne outbreak of cryptosporidiosis occurred in an apartment complex, and tap water samples were collected and examined. It was found that the main drinking water source was polluted with sewage from a septic tank in the apartment complex[54]. On the other hand, the investigators detected *Cryptosporidium* oocysts in all water tanks (100%) in Alexandria city, Egypt using flow cytometry[55]. Another research was conducted also in Alexandria to study cryptosporidiosis among children in urban and rural areas. The investigators collected 30 stored water samples from each area and *Cryptosporidium* oocysts were found in only 2 samples (6.7%) in urban area and 4 samples (13.3%) in the rural area[56]. Abundance of *Cryptosporidium* oocyst in the present work may be greatly affected by zoonotic contamination as most tanks were opened making them more liable to rodent feces shown to be reservoir host for *Cryptosporidium* spp.[57,58].

The results of the present study showed that *Giardia* cysts were detected in 13.7% of all water samples, 12.3% from tap water and 16.7% from water tanks. In Egypt, *Giardia* cysts were detected in different sources of potable water samples with a lower rate (2.1%) in Dakahlia governorate[52] and 7.4% in tap water samples in Gharbiya governorate[43]. Higher detection rates were reported on examination of different water sources in Argentina (31%)[47] and 41.7% in Brazil[44], while a similar result

(15.4%) was recorded in Iran [45]. In a study conducted in Addis Ababa, Ethiopia, the investigators reported a low detection rate in tap water (1%) and a higher rate (29%) in treated water storage tanks[59]. Using other methods for detection of *Giardia* cysts, the investigators employed flow cytometry to identify and evaluate *Giardia* cysts viability in water samples in Assuit and recorded 29.2% detection rate[60]. Use of PCR allowed investigators to detect and genotype 8.4% of *Giardia* in Portugal[59] and 33.8% in Spain[51]. Using IFA, detection rates of 9.6% and 45.6% were reported in Russia[61] and Spain[51], respectively. In the Russian study, the investigators attributed the lower detection rate to the low sensitivity of the immunofluorescent staining methods used. It is worthwhile to add that 7.2% of the examined water samples were contaminated by both *Cryptosporidium* oocysts and *Giardia* cysts, using PCR[50].

Our study had two important limitations; first, we didn't examine the water sources for other pathogenic protozoa (*Entamoeba* spp.), as well as emerging protozoa such as *Blastocystis* spp., *Cyclospora cayetanensis, Dientamoeba fragilis* and *Isospora belli*. Second is the inability to conduct molecular techniques (PCR) to identify *Naegleria* spp. However, it was concluded that 89.2% of all our water sources in Fayoum governorate possess a high risk to our community. Therefore, the application of adequate operational practices combined with the promotion of source water protection programs will ensure the effectiveness of current conventional treatment processes in preventing protozoan cysts/oocysts, FLA and other microbial contaminants from entering the final water supply, thus ensuring the continuous provision of safe drinking water to consumers.

CONFLICT OF INTEREST

There are no conflicts of interest.

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