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Surveillance of Potentially Pathogenic Free-Living Amoebae through Drinking Water Treatment Processes in Fayoum Governorate, Egypt

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ABSTRACT

Free-living amoebae (FLAs) are a large diverse group of unicellular organisms in the kingdom protozoa. The aim of this study was to observe the existence of free-living amoebae through drinking water treatment plants in Fayoum governorate, Egypt. Water samples were collected from 4 drinking water treatment plants (DWTPs) and filtered through nitrocellulose membranes, then placed on non-nutrient agar with *Escherichia coli* for cultivation of free-living amoebae. The obtained amoebae were morphologically identified and molecularly confirmed to genus level. The results revealed that the occurrence of free-living amoebae in intakes, finished water and distribution systems of the examined DWTPs reached 72.9, 25 and 37.5 %, respectively. The removal percentage of free-living amoebae through different treatment processes reached its highest rate in New Azab DWTP (72.7%), followed by Old Azab DWTP (70.%), while it was the same in each of Old Kohafa and New Kohafa DWTPs reaching 57.1%. Almost all the morphologically identified *Acanthamoeba* strains (98.5%) proved to be related to genus *Acanthamoeba* when tested by PCR, while 64.6% of the morphologically identified *Naegleria* strains proved to be related to genus *Naegleria* by PCR and no *Balamuthia mandrillaris* amoebae were detected whether morphologically or by PCR. In conclusion, the presence of free-living amoebae in drinking water exerts an indirect public health hazards as they may harbor pathogenic microorganisms that can escape drinking water treatment processes and reach to end user.

Key words: Free-living amoebae, Morphology, PCR, Drinking water, Fayoum governorate

Introduction

Free-living amoebae (FLAs) are a large diverse group of unicellular organisms in the kingdom protozoa. Although these organisms have a common amoeboid motion, i.e. crawling-like movement, they have been classified into several different groups; the majority of these groups are free-living while few members are parasitic (Cavalier-Smith, 1993).

Pathogenic and opportunistic free-living amoebae such as *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri* and *Sappinia diploidea* are aerobic mitochondriate eukaryotic protists that occur world-wide and can potentially cause infections in humans and other animals (Schuster and Visvesvara, 2004; Visvesvara and Maguire, 2006).

Free-living amoebae feed on microorganisms present on wet surfaces, in diverse environments (Brown and Barker, 1999) and even at the air–water interface (Preston *et al*., 2001). The manner of *Acanthamoeba* movement is similar both at solid substrata and water-air interface. Adhesion forces developed between *Acanthamoeba* and the water-air interface are greater than gravity, and thus amoebae are also transported passively without detachment from the water surface (Preston *et al*., 2001). Pseudopodia of trophozoites may be used to capture food particles, which are usually bacteria (Weekers *et al*., 1993), but algae, yeasts (Allen and Dawidowicz, 1990) and other protists are also grazed upon. Food uptake occurs by phagocytosis and pinocytosis.

Disinfectants such as chlorine, chlorine dioxide, deciquam 222, and ozone possess amoebicidal properties and would be suitable for the disinfection of waters contaminated with pathogenic FLAs. However, the ultimate choice of a particular disinfectant remains closely tied to the chemical and physical characteristics of the water to be treated and the particular properties of the disinfectant. Chlorine was introduced into water treatment nearly 80 years ago (White, 1972). With a batch system, in which only a single dose of chlorine is

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added, initial concentrations of 0.74, 0.79, 1.0, and 1.25 mg-liter-' had a sterilizing effect on *N. fowleri, N. gruberi, A. castellanii*, and *A. culbertsoni*, respectively. The use of chlorine as a disinfectant for pathogenic FLAs was reported by Anderson and Jamieson (1972), who failed to eliminate *N. fowleri* which had been super-chlorinated with 10.0 mg of chlorine per liter. With a batch method, Derreumaux *et al.* (1974) demonstrated that water with an initial content of 1.4 mg of chlorine per liter was able to sterilize 2 x 10^3 trophozoites of both *Naegleria* and *Acanthamoeba* per cm^3 in 30 min. They also reported that trophozoites of *Naegleria* were more sensitive to chlorine than those of *Acanthamoeba*. It was also observed that 10^3 cysts of *Naegleria* per cm^3 were sterilized by 2 mg of chlorine per liter in 30 min, whereas 40 mg of chlorine per liter failed to sterilize cysts of *Acanthamoeba* (De Jonckheere and van de Voorde, 1976).

Members of only four genera of FLAs are opportunistic pathogens causing infections of the central nervous system, lungs, sinuses and skin, mostly in immunocompromised humans. *Balamuthia* is also associated with disease in immunocompetent children, and *Acanthamoeba* spp. cause a sight-threatening infection, *Acanthamoeba* keratitis, mostly in contact-lens wearers. *Naegleria fowleri* causes an acute and fulminating meningoencephalitis in immunocompetent children and young adults. Because only one human case of encephalitis caused by *Sappinia diploidea* is known, generalizations about the organism as an agent of disease are premature (Visvesvara *et al.*, 2007). *Acanthamoeba* can also cause central nervous system infections, including gorillas, monkeys, dogs, ovines, bovines, horses, and kangaroos, as well as birds, reptiles, amphibians, and fishes (Martinez and Visvesvara, 1997; Dykova *et al*., 1999).

So, the objective of the present work was to evaluate the occurrence of potentially pathogenic free-living amoebae in four drinking water treatment facilities and the efficacy of conventional drinking water treatment steps for the removal of free living amoebae from the produced drinking water.

Material and Methods

Samples and sampling sites

Water samples were collected from four drinking water treatment plants (DWTPs) located in Fayoum governorate. This governorate is bounded from the east, west and north by Giza governorate, while its southern boundary is Beni Suef governorate (Figure 1). Three DWTPs namely: New Azab, Old Azab and Old Kohafa are working with rapid sand filtration system, while only one DWTP namely New Kohafa and was operated by slow sand filtration system.

Three types of water were collected from the four mentioned DWTPs: raw (inlet) water, finished (outlet) water and tap water from the distribution system. Only in case of New Azab DWTP and in addition to the three types of water samples, other three water samples were collected: pre-chlorinated raw water, clarified water and filtered water.

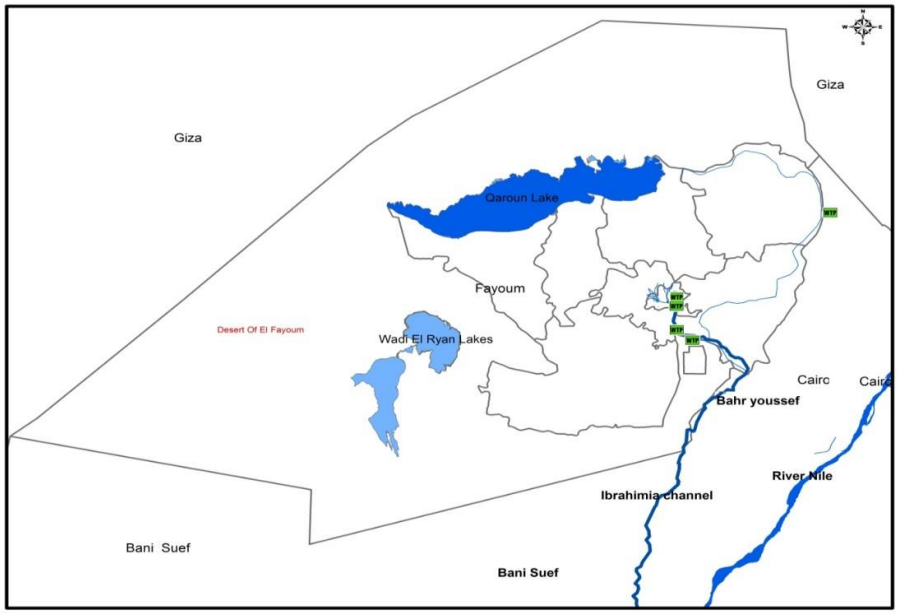

Fig. 1: Diagrammatic map for the examined DWTPs in Fayoum governorate

Water samples (one liter volume each) were collected monthly from each of the previously mentioned four DWTPs. The samples were separately collected in one liter volume autoclavable polypropylene Stoppard containers. Water samples were collected monthly from each water type along one year period from March 2010 to February 2011.

Concentration, cultivation and isolation of FLAs

Collected water samples were separately concentrated using cellulose nitrate membranes (0.45µm pore size and 47mm diameter) that were then placed on non-nutrient agar plates spread with *Escherichia coli* bacteria and incubated at 37°C for one week with daily microscopic examination for the presence of amoebic growth according to the method of Page (1974). Amoebic plaques on positive plates were separately sub-cultured for isolation of and propagation of different amoebic isolates as the method described by Al-Herrawy (1992).

Morphological identification of isolated FLAs

Isolated and purified freshwater amoebae were identified on the bases of both trophozoite and cyst morphological characteristics (Pussard and Pons, 1977; Page, 1988).

Molecular characterization of isolated FLAs using PCR

A simple PCR technique was used, consisting of DNA extraction and amplification followed by agarose gel electrophoresis. DNA was extracted from the obtained amoebae using lysis buffer containing 2% CTAB as the method described by Winnebenninckx *et al.* (1993). Genus-specific primers and their fragments of target genes used for detecting *Acanthamoeba*, *Naegleria* and *Bulamutia mandrillaris* were displayed in Table 1. PCR reaction and its additives were carried out according to the method of Kilic *et al.* (2004). Electrophoresis on agarose gel was done to separate DNA fragments as described by Helling *et al.* (1974).

Table 1: Description of the primers used in PCR for *Acanthamoeba, Naegleria* and *Bulamutia mandrillaris.*

Pathogen	Primer sequence (5´- 3´)	Amplification size (bp)	References
Acanthamoeba spp.	tttgaattcgctccaatagcgtatattaa tttgaattcagaaagagctatcaatctgt	910-1170	Kilic *et al.* (2004)
Naegleria spp.	gaacctgcgtaggatcattt tttcttttcctcccctttatta	409	Pelandakis *et al.* (2000)
Bulamutia mandrillaris	cgcatgtatgaagaagacca ttacctatataattgtcgatacca	1075	Booton *et al.* (2003)

Statistical analysis

The obtained data were statistically analyzed using Paired T-Test through Minitab statistical program (Mayer and David, 2004).

Results

Occurrence of FLAs in intakes of the examined DWTPs

In general, the occurrence of free-living amoebae in intakes of the examined DWTPs reached 72.9%. The highest occurrence of free-living amoebae (91.7 %) was recorded in the intake of Old Azab DWTP, followed by intake of New Azab DWTP (83.3%). On the other hand, the lowest occurrence of free-living amoebae was recorded both in intakes of Old Kohafa and New Kohafa DWTPs (58.3% for each). (Table 2).

In general, the occurrence of free-living amoebae in finished water reached 25%. The same occurrence of free-living amoebae (25%) was recorded in finished water of all examined DWTPs. Also, there was no difference between occurrence of FLAs after rapid sand filtration (in New Azab, Old Azab and Old Kohafa DWTPs) and slow sand filtration (In New Kohafa DWTP) (Table 2).

The occurrence of free-living amoebae in distribution system of Fayoum governorate reached 37.5%. The highest occurrence of free-living amoebae (41.7 %) was recorded in the distribution system of New Azab DWTP, followed by distribution system of Old Azab (33.3%), while the lowest occurrence was recorded in distribution systems of Old Kohafa and New Kohafa (8.3% for each). (Table 2).

Statistically, there was no significant difference in the prevalence of FLAs in outlets versus distribution by paired t-test as P. value = 0.824 (i.e. > 0.05) (Table 3).

Efficiency of DWTPs for the removal of FLAs

It was found that the removal percentage of free-living amoebae through different treatment processes reached its highest rate in New Azab DWTP (72.7%), followed by Old Azab DWTP (70.%). On the other hand, the capability of Old Kohafa and New Kohafa DWTPs in removing free-living amoebae through drinking water treatment processes was equal as each of them could remove 57.1% of free-living amoebae present in their intake water (Table 4 and figure 2).

Statistically, the removal of FLAs was significantly effective through drinking water treatment processes by DWTPs, as P. value = 0.011 (i.e. < 0.05) (Table 5).

It was also observed that drinking water treatment processes were very significantly effective in removal of FLAs from the produced potable drinking water. Also, the distribution system did not have a bad effect on the quality of the drinking water, as P. value = 0.001 (i.e. < 0.05) (Table 6).

Table 2: Occurrence of free-living amoebae in the four examined DWTPs.

DWTPs	Total number of examined samples	Amoebae-positive samples on NN agar					
		Inlets		Outlets		Distribution systems	
		No.	%	No.	%	No.	%
New Azab	12	10	83.3	3	25.0	5	41.7
Old Azab	12	11	91.7	3	25.0	4	33.3
New Kohafa	12	7	58.3	3	25.0	1	8.3
Old Kohafa	12	7	58.3	3	25.0	1	8.3
Total	48	35	72.9	12	25.0	18	37.5

Table 3: Paired T for occurrence of FLAs in outlets versus distribution in DWTPs

	N	Mean	St Dev	SE Mean
Inlets	4	9.50000	1.29099	0.64550
Outlets	4	3.00000	1.63299	0.81650
Difference	4	6.50000	2.64575	1.32288

95% CI for mean difference: (2.29002; 10.70998), T-Test of mean difference = 0 (vs not = 0): T-Value = 4.91 P-Value = 0.016

Table 4. Efficiency of DWTPs for the removal of FLAs .

DWTPs	FLAs (occurrence %)		FLAs (removal %)
	Intake	Finished water	
New Azab	91.7	25.0	72.7
Old Azab	83.3	25.0	70.0
New Kohafa	58.3	25.0	57.1
Old Kohafa	58.3	25.0	57.1

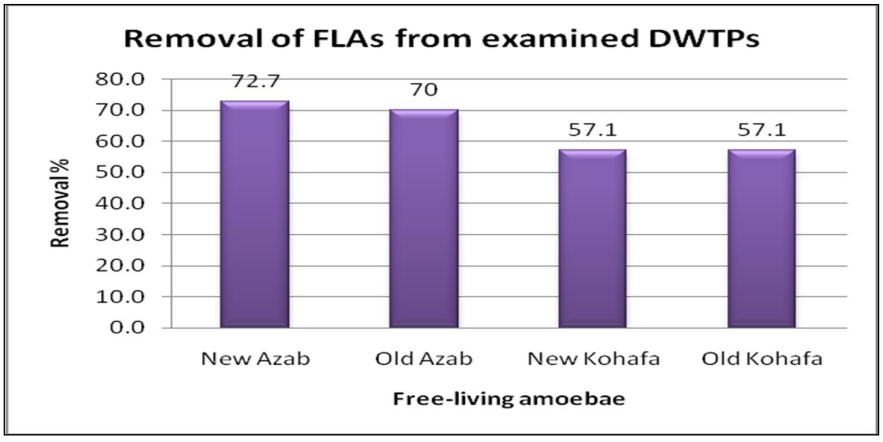

Fig. 2: Efficiency of the 4 examined DWTPs for the removal of FLAs.

Molecular confirmation of some strains of the morphologically identified FLAs

The morphologically identified FLAs were subjected to molecular confirmation by simple PCR techniques using genus specific primers for *Acanthamoeba* and *Naegleria* as well as species specific primers for *Balamuthia mandrillaris.*

Almost all the morphologically identified *Acanthamoeba* strains (98.5%) proved to be related to genus *Acanthamoeba* when they were tested by PCR. About 64.6% of the morphologically identified *Naegleria* strains

proved to be related to genus *Naegleria* by PCR. No *Balamuthia mandrillaris* amoebae were detected whether morphologically or by PCR (Table 7 and figure 3).

Also, all the morphologically identified *Acanthamoeba* species (that were similar to and suspected to be *Balamuthia*) gave negative results when tested with PCR using species specific primers for *Balamuthia mandrillaris* (Table 7).

Table 5: Paired T for removal of FLAs in inlets versus finished water.

	N	Mean	St Dev	SE Mean
Inlets	4	8.75000	2.06155	1.03078
Finished	4	3.00000	0.00000	0.00000
Difference	4	5.75000	2.06155	1.03078

95% CI for mean difference: (2.46961; 9.03039), T-Test of mean difference = 0 (vs not = 0): T-Value = 5.58, P-Value = 0.011

Table 6. Paired T for removal of FLAs in inlets versus distribution**.**

	N	Mean	St Dev	SE Mean
Inlets	4	8.75000	2.06155	1.03078
Distribution	4	2.75000	2.06155	1.03078
Difference	4	6.00000	0.81650	0.40825

95% CI for mean difference: (4.70077; 7.29923), T-Test of mean difference = 0 (vs not = 0): T-Value = 14.70; P-Value = 0.001

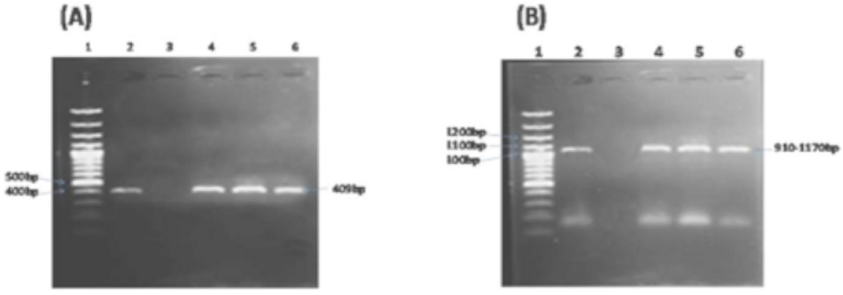

Fig. 3: Agarose gel electrophorisis for PCR amplified product of DNA from:

(A) Naegleria spp. Lane 1: Marker; Lane 2: control Positive; Lane 3: control negative; Lane 4,5,6: positive samples.
(B) Acanthamoeba spp. Lane 1: Marker; Lane 2: control Positive; Lane 3: control negative; Lane 4,5,6: positive samples.

Table7: PCR positive samples for free-living amoebae in different sampling sites of the examined DWTPs.

Sampling sites	Amoebae-positive samples on NN agar	Amoebae-positive samples by PCR					
		Acanthamoeba		*Naegleria*		*Balamuthia mandrillaris*	
		No.	%	No.	%	No.	%
Inlets of DWTPs	35	34	97.1	26	82.9	0	0
Finished water	12	12	100	3	25.0	0	0
Distribution system	18	18	100	13	72.2	0	0
Total	65	64	98.5	42	64.6	0	0

Discussion

Being cyst-formers, many amoebae are able to survive for long periods under extreme conditions of temperature, pressure, humidity, altitude, solar radiation and gaseous pollutants (Dimmick *et al.*, 1979; Lundholm, 1982). Survival of amoebae in the air depends on their morphophy-siological features, micrometeorological and physico-chemical conditions, and on the interaction between these parameters (Cox, 1987).

In the present work, the highest occurrence of free-living amoebae in water samples collected from the intake (raw) water samples reached 91.7 in Old Azab DWTP, followed by 83.3, 58.3 and lastly 58.3% in New Azab, Old Kohafa and new Kohafa DWTPs, respectively. Other workers in Egypt (Hilali *et al.*, 1994; Al-Herrawy *et al.*, 2013) recorded higher occurrences of free-living amoebae in raw water samples from Cairo (87.5%) and Behera (100%) governorates, respectively. On the other hand, Hamadto *et al.* (1993) also in Egypt detected free-living amoebae in a very lower incidence (20%) in freshwater. In our opinion, these variations might be due to differences in the locality as well as date of sampling. In other countries of the world, other workers recorded also a lower incidence of free-living amoebae (43.3%) in freshwater samples collected from James River, USA (Ettinger *et al.*, 2003). In Bulgaria, Tsvetkova *et al.* (2004) recorded freshwater amoebae also

in a lower percentage (61.1%) than that recorded in the present study that might be attributed to the lower atmospheric temperature in those countries.

In the present study, free-living amoebae were detected in 25% of the finished (outlet) water samples collected from the 4 examined DWTPs of Fayoum governorate. Also in Egypt, Shaban (2013) recorded similar results (25%) but in the finished water of Damanhour DWTP in Behera governorate. A higher occurrence of free-living amoebae was recorded in 371 (79%) samples from household distribution systems of Ohio, USA (Lauren *et al.*, 2011).

In the present study, the highest occurrence of free-living amoebae in distribution systems (41.7%) was detected in New Azab DWTP, followed by 33.3, 3.8 and 3.8% in Old Azab, Old Kohafa and New Kohafa DWTPs, respectively. Free-living amoebae were recorded in a lower occurrence (4%) (Hamadto *et al.* 1993), while a nearly similar percentage (23.6%) of FLAs was detected by Hilali *et al.* (1994). Other workers in the UK also detected free-living amoebae from household tap water samples in a higher percentage (89%) (Kilvington *et al.*, 2004). In Korea, Jeong and Yu (2005) recorded freshwater amoebae in domestic tap water samples in a percentage (46.9%) higher than that recorded in the present study. Other studies within the USA and the UK found amoebae in 19% and 48% of households of healthy subjects, respectively (Seal *et al.* 1992; Shoff *et al.* 2008). These other studies contained fewer samples per house than were examined in our study. The standardized collection of both water and biofilm swab samples by plumbing technicians in this study, rather than collection by the home resident as done in the previous US study (Shoff *et al.* 2008), might have resulted in more consistent sample quality and, as a result, increased detection limits of FLAs. There are factors known to affect the presence of FLAs, such as water source, water treatment method and geographic location, and differences in these across the USA limits the confidence with which these results can be applied to other regions (Lauren *et al.*, 2011).

Concerning elimination of free-living amoebae during drinking water treatment process in the present work, it was found that the highest removal efficiency was recorded in New Azab DWTP (72.7%), followed by Old Azab DWTP (70%), while both New Kohafa and Old Kohafa DWTPs recorded the same removal efficiency representing 57.1% for each. Other workers in Egypt, recorded that the treatment processes applied to different stages of drinking water treatment production in Damanhour DWTP, Behera governorate, could remove 75% of FLAs present in the inlet water (Al-Herrawy *et al.*, 2013; Abu Kabsha, 2013). This record was higher than that of the present study, although all inlet samples of Damanhour DWTP harbored FLAs. In our opinion, the higher removal of FLAs from Damanhour DWTP was an indication that treatment steps were efficiently processed than in the examined DWTPs of Fayoum governorate.

Since chlorine was introduced into water treatment nearly 80 years ago, it has become almost the only method used for the active disinfection of potable water supplies (White, 1972). This predominant position of chlorine has been gained because of its potency and range of effectiveness as a germicide; its ease of application, measurement, control, and economy; its relative freedom from toxic or physiological effects; and its reasonable persistence in waters. It is thought that the difference in susceptibility to chlorine between the two genera (*Naegleria* and *Acanthamoeba*) is more likely to be a consequence of the difference in chemical (especially protein) composition of the cell membranes than of the difference in metabolism. Recently, it was reported that the Ct values (in mg min/L) required for 2-log reduction of *Acanthamoeba, Naegleria* and *Hartmannella* cysts treated with chlorine reached 865, 29 and 156, respectively. On the other hand, the same organisms (Dupuy *et al.*, 2014). In our opinion this study declared that the usually used chlorine doses (2-7 mg/L) for disinfection of the produced water in drinking water treatment plants were ineffective for killing free-living amoebae.

In the present study, it was found that *Acanthamoeba* species were the most prevalent free-living amoebae in the examined water samples. It has been established previously that *Acanthamoeba* spp. were the most common and opportunistic amphizoic protozoa (Page, 1988; Schuster and Visvvesvara, 2004). These organisms have gained medical importance since some of them can produce pathologies in humans such as amebic encephalitis (Marciano-Cabral *et al.*, 2000) and amebic keratitis, a sight threatening ulceration of the cornea (Niederkorn *et al.,* 1999; Sharma *et al.*, 2000).

Although there are some differences in sizes and pseudopod structure of the trophozoites, *Acanthamoeba* spp look remarkably similar when viewed under the microscope, but careful observation (on the cyst stage especially) led to a number of species being described and a classification system created (Pussard and Pons 1977). This scheme has fallen out of favor with some workers as a result of the realization that cyst morphology depends on the conditions in which they were created (Stratford and Griffiths 1978), and the advent of PCR. Many strongly suspected that the twenty or so species named based on morphology was unlikely to be valid and attempts were made to use isoenzyme analysis as a means to classify the genus (Costas and Griffiths 1984). These efforts largely failed to make the situation clearer, but one promising study did at least identify *A. jacobsi* and *A. lenticulata* as being separate and distinct groups (Flint *et al.* 2003). These species are also currently the only valid named species being T15 and T5 respectively.

In the present study, the morphologically identified free-living amoebae were subjected to molecular confirmation by simple PCR techniques using genus specific primers for *Acanthamoeba* and *Naegleria* as well as species specific primers for *Balamuthia mandrillaris.* Almost all the morphologically identified *Acanthamoeba* strains proved to be related to genus *Acanthamoeba* when they were tested by PCR. Other workers in Egypt found that 94.9% of microscopically *Acanthamoeba* +ve swimming pool samples were also positive by using PCR technique (Al-Herrawy *et al.*, 2014). In another work, also conducted in Egypt, it was noticed that all water samples proved to be microscopically +ve for *Acanthamoeba* were also confirmed by PCR to be related to genus *Acanthamoeba* (Abu Kabsha, 2013). Other workers in Egypt morphologically detected six *Acanthamoeba* species (namely A. *polyphaga*, A.*castellanii*, A. *rhysodes*, A. *mauritaniensis*, A. *royreba* and A. *triangularis*) isolated from the examined swimming pool water in Cairo. All the identified species of *Acanthamoeba* were molecularly confirmed to be related to the genus *Acanthamoeba* (Al-Herrawy *et al.*, 2014). They also concluded that the culture method was cheaper and easier than PCR techniques that were faster for the detection of free-living amoebae.

In the present work, the predominance of *Acanthamoeba* species in almost all the examined water types might be due to their ability to live in all water types, to tolerate weather changes and low humidity. Mazur *et al.* (1995) demonstrated that cysts of *Acanthamoeba* retained viable amoebae for over 24 years after storage in water at 4°C.

In the present study, about 82.9% of the morphologically identified *Naegleria* strains, isolated from raw water samples, proved to be related to genus *Naegleria* by PCR. Other workers in Egypt found that the recovery percentage of *Naegleria*-positive PCR samples reached 83.6% (a nearly similar that of the present work) in the microscopically-positive isolates from Nile water (Hikal, 2010). Other workers also in Egypt recorded a slightly lower percentage of *Naegleria* (80%) by PCR in the 20 culturally-positive Nile water samples for *Naegleria* (Afew, 2014). In Belgium, members of genus *Naegleria* were recorded by PCR in 80% of water samples collected from lakes and ponds (Declerck *et al.,* 2007). Other workers recorded members of genus *Naegleria* by PCR in rivers in Belgium (Behets *et al.*, 2003), Switzerland (Gianinazzi *et al.*, 2009), Venezuela (Cermeño *et al.*, 2006) and Germany (Hoffmann and Michel, 2001) in greatly lower percentages than that recorded in the present study, reaching 56.4, 27.7, 44.4, and 11.1%, respectively . In Taiwan, Huang and Hsu (2010) detected *Naegleria* spp. by PCR in 14.2% recreational water samples. On the other hand, Ithoi *et al.* (2011) in Malaysia recorded a higher percentage of *Naegleria* (100%) in the 33 microscopically-positive isolates from recreational water.

In the present investigation, the prevalence of *Naegleria* by PCR reached 100% in the 3 culturally-positive tap water samples for *Naegleria.* Other workers in Egypt detected *Naegleria* amoebae by PCR in a slightly lower percentage (91.7%) in microscopically-positive tap water samples (Hikal, 2010). Other workers in Spain (Garcia *et al.*, 2013) detected *Naegleria* amoebae in 31.7% of total examined tap water samples. In Australia, Dorsch *et al.* (1983) established that the presence of *Naegleria* was associated with the unusually high summer temperatures and inadequate chlorine residuals.

In the present work, amoebae belonging to genus *Balamuthia* did not detected in the examined water samples neither by morphological criteria nor by PCR techniques. To the best of our knowledge, amoebae of genus *Balamuthia* did not isolated from the Egyptian aquatic environment till now. Other workers in other countries have only been able to isolate *B. mandrillaris* from the environment on four occasions (twice from Californian soils, once from dust in Iran and the last from soil in Peru) (Schuster *et al.*, 2003; Dunnebacke *et al.*, 2004; Cabello-Vílchez *et al.*, 2014).

In the present study, members of genus *Acanthamoeba* were the predominant free-living amoebae in almost all the collected water samples. It is obviously known that most of *Acanthamoeba* species are incriminated as agents of diseases in humans and even animals (Marciano-Cabral and Cabral, 2003). Because GAE caused by *Acanthamoeba* spp. occurs in hosts with weakened immune functions, no clearly defined methods exist for the prevention of infection with these amoebae. In the case of AK, however, because contact lenses and lens care solutions are well-known risk factors, educating lens wearers regarding the proper care of contact lenses (and contact-lens cases) is important for the prevention of infection. Moreover, lens wearers should be instructed not to wear contact lenses during swimming, performing water sport activities, or relaxing in a hot tub or Jacuzzi (Sriram *et al.*, 2008). Other studies reported that five different genera were identified concerning FLAs diversity. The most frequently identified genus in tap water was *Acanthamoeba*, although it must be considered that these studies mostly used morphology to identify FLAs. As *Acanthamoeba* spp. have a distinctive polyhedral double walled cyst (Visvesvara, 1991) they are more readily identified. The next three most frequently identified genera in order were *Hartmannella*, *Vahlkampfia* and *Vannella*.

In conclusion, the presence of potentially pathogenic *Naegleria* and *Acanthamoeba* species in drinking water may lead to disorders for the users. Moreover, the presence of other free-living amoebae in drinking water exerts an indirect public health hazards as they may harbor pathogenic microorganisms that can escape drinking water treatment processes and reach to end users.

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