Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(4): 627 – 637 (2019)
www.ejabf.journals.ekb.eg

Detection and removal of free-living amoebae in two different facilities for drinking water by culture and PCR

Ahmad Z. Al-Herrawy[1]*; Mohamed A. Marouf[1]; Nora R. Zahglol[2] and Mahmoud A. Gad[1]

1- Environmental Parasitology Laboratory, Water Pollution Research Dept., National Research Centre, 12622 Dokki, Giza, Egypt.
2- Microbiology Laboratory, Fayoum Water and Wastewater Holding Company, Fayoum governorate, Egypt.

* Corresponding author: alherrawy@gmail.com

ARTICLE INFO
Article History:
Received:Oct. 14, 2019
Accepted: Nov. 12,2019
Online: Nov.22, 2019

Keywords:
Amoebae
Microscopy
PCR
Drinking water
treatment plant
compact unit

ABSTRACT

Conventional drinking water treatment plants (CDWTPs) and compact units (CUs) are the main 2 types of drinking water treatment using freshwater as a source for drinking water in Egypt. The Egyptian standards for drinking water denied the presence of any type of living protozoa in drinking water produced for human use.

In the present study, raw and finished water samples were separately collected from a CDWTP and a CU in Giza governorate, Egypt. Samples were separately concentrated through nitrocellulose membrane filters (0.45µm pore size). Potentially pathogenic free-living amoebae (FLAs) were detected in the concentrates by cultivation and PCR.

By culture and microscopy, five genera of free-living amoebae (*Acanthamoeba*, *Vahlkampfia*, *Hartmannella*, *Naegleria* and *Vannella*) were encountered from inlet water samples of the 2 treatment systems. Obtained data declared that 87.5% and 91.7% of inlet water from CDWTP and CU, respectively contained potentially pathogenic FLAs that were also isolated from 20.8% and 45.8% of finished water samples from CDWTP and CU, respectively. Of the isolated FLAs from inlet water samples, *Acanthamoeba* were detected in finished water samples of both CDWTP and CU, while *Hartmannella* were isolated from finished water of only CU. Removal of FLAs reached 76.2% in CDWTP, while it reached only 50% in CU. Molecularly, only three genera were encountered representing *Acanthamoeba*, *Hartmannella* and *Naegleria* but with a lower incidence than that revealed by the culture method.

In conclusion, inlet water samples from both CDWTP and CU contained potentially pathogenic FLAs. Although CDWTP was more effective than CU in removing free-living amoebae, still some of these organisms could be detected in finished water and thus cause health risk hazards to consumers.

INTRODUCTION

Safe and clean drinking water is considered one of the human right essentials. Waterborne diseases caused by biological agents, like pathogenic protozoa, bacteria, viruses and helminthes, are the most common and widespread health risks associated with drinking water (WHO, 2011). Protozoa represent an extremely diverse group of unicellular organisms; some of them are considered problems for the water industry.

Moreover, they are resistant to inactivation by chemical disinfectants used in drinking water act. These parasites generally cause diarrhea and gastroenteritis of varying severity, although more serious consequences (including death) can occur (APHA, 2018).

In the United States, 18% of drinking water-associated outbreaks between 1971 and 2006 were caused by protozoa (Craun *et al.,* 2010). Of the 325 water associated protozoan disease outbreaks reported worldwide, *Acanthamoeba* spp., and *Naegleria fowleri* were associated with 0.3% of outbreaks, but a definitive association between drinking water and disease outbreaks has yet to be established for some of these organisms (APHA, 2018). Free-living amoebae are distributed everywhere in the environment (Gill and Fast, 2006; Anjum *et al*., 2019).

A conventional drinking water treatment plant (CDWTP) consists of 4 different steps (WHO, 2004), beginning with the intake water (raw surface water). Raw water from the intake is sucked in pipes having coarse metal sieves with 4cm pore size for prevention of coarse objects from getting entrance with sucked water. The sieved raw water is pumped to coagulation and precipitation basins where it is mixed with aluminum sulfate to aid in the flocculation and precipitation of the debris and microorganisms found in raw water. After that, the clear water on the top of sedimentation basins is collected and passed on sand filters to get rid of the remaining microorganisms as well as escaped very small particulates. Filtered water is then collected in storage tanks where it is injected with chlorine dose of 2mg/liter for disinfection. The disinfected water (outlet water) is ready to be pumped and distributed to the consumers as a drinking water (WHO, 2004).

The major two types of drinking water treatment plants are conventional drinking water treatment plant (CDWTP) and compact unit (CU). Conventional drinking water treatment plants produce larger amounts of water compared to compact units, so they were widely used in large municipal water systems by the 1920s. Drinking water systems having rapid sand filters use relatively coarse, sand and other granular media to remove impurities and particles that have been trapped in flocs through the use of chemicals (typically alum) for flocculation. After flocculation step, the unfiltered water flows through the filter medium under pumped pressure and the flocs are trapped in the sand matrix (WHO, 2004). Compact units consist of consequent treatment chambers ending with the production of drinking water. Typical CU 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 are available to supply drinking water for 100 to 25,000 persons per unit (https://www.rwlwater.com/compact-water-treatment-plants/).

The most commonly used disinfection process is chlorination. Ozonation, ultraviolet irradiation, chloramination and application of chlorine dioxide are also used. These methods are very effective in killing bacteria and can be reasonably applied for inactivation of viruses (depending on type), and some may inactivate trophic stages of protozoa. For effective removal or inactivation of protozoan cysts and oocysts, filtration with the aid of coagulation and flocculation (to reduce particles and turbidity) followed by disinfection (by one or a combination of disinfectants) are the most practical methods (WHO, 2011).

It is essential that an overall management strategy is implemented in which multiple barriers, including source water protection and appropriate treatment processes, as well as protection during storage and distribution, are used in conjunction with disinfection to prevent or remove microbial contamination (WHO,

2011). Over 600 cases of amoebic encephalitis caused by pathogenic free-living amoebae have been reported worldwide and in Japan, 24 cases have been reported from the first case in 1976 up to 2018. Therefore, encephalitis caused by pathogenic free-living amoebae should be added to the differential diagnosis of encephalitis patients (Hara *et al*., 2019). So, the aim of the present work is to compare between two different drinking water treatment facilities (CDWTP and CU) for the removal of potentially pathogenic FLAs.

METHODOLOGY

Samples and sampling sites

Water samples were collected from two different drinking water treatment facilities (a conventional drinking water treatment plant and a compact unit) in Giza district, Egypt. Four different sampling locations namely: a) Inlet (surface or freshwater) of a conventional drinking water treatment plant; b) Outlet (finished or completely treated drinking water) of a conventional drinking water treatment plant; c) Inlet (surface or freshwater) of a compact unit; d) Outlet (finished or completely treated drinking water) of a compact unit.

Water samples were collected for a one year period (from February 2017 to January 2018) for the detection of potentially pathogenic FLAs. Collected water samples were transported in ice-box at 4-8^{o}C to the laboratory at the same day of collection (HPA, 2004; ISO/FDIS, 2006).

Water samples (one litre from each sampling site) were separately collected in sterile polypropylene containers and used for the detection of potentially pathogenic FLAs.

Concentration and culturing of FLAs

One litre of each water sample was collected in a sterile autoclaved plastic bottle and used for the detection and cultivation of potentially pathogenic free-living amoebae. The sterile one litre of each sample was filtered under a sterile condition through 0.45 µm nitrocellulose membrane (47mm in diameter) using stainless steel vacuum filter holder (Sartorius) and then the membrane was placed face to face on non-nutrient (NN) agar plate covered with dead *Escherichia coli* and incubated at 37ºC for one week with daily microscopic examination to detect genera of FLAs **(Page, 1988)**.

Molecular detection of FLAs

The surfaces of NN agar plates, cloned with free-living amoebae, were separately washed with sterile PBS buffer and the washing solution (containing FLAs) was then centrifuged at 250xg for 5-10min. Extraction of DNA from amoebae was performed using a MagaZorb® DNA Mini-Prep Kit (Madison, USA) according to the manual instructions.

The PCR reactions were separately done using different primer pairs for the previously detected *Acanthamoeba, Naegleria*, *Vermamoeba, Vahlkampfia* and *Vannella* isolates by culture method (Table 1).

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) (Table 1), and 7.5µl nuclease-free water. The thermal profile for each protozoan was shown in Table 2.

Electrophoresis was carried out in a horizontal electrophoresis system (Power Pac Basic, Bio-Rad, Munich, Germany) at 120 volts for 20min using gels composed

of 1.6% agarose, 1% TAE buffer and 5µL of RedSafe™ Nucleic Acid Staining Solution (Intron Biotechnology, Korea) per 100mL. The results were visualized under UV radiation (PCI-Gel-Imager, Intas, Göttingen, Germany). The obtained data were statistically analyzed by paired t-test and two samples t-test using Minitab statistical program (Minitab Inc., Pennsylvania, USA). P values less than 0.05 were considered significant.

Table 1: Specific primers used for molecular detection of tested free-living amoebae

Parasite name	Primer name	Primer sequence (5`-3`)	Target genome	Product length(bp)	Reference
Acanthamoeba spp.	AcantF900	Cccagatcgtttaccgtgaa	18S r RNA gene	About 180	**Qvarnstrom *et al.*, 2006**
	AcantR1100	taaatattaatgccccc aactatcc			
Naegleria spp.	Nae3-For	Caaacaccgttatgacaggg	18S r RNA gene	183	**Schild *et al*., 2007**
	Nae3-Rev	Ctggtttcccttaccttgcg			
Hartmannella vermiformis	Hv1227F	Ttacgaggtcaggacactgt	18S rRNA gene	502/503	**Kuiper *et al*., 2006**
	Hv1728R	Gaccatccggagttctcg			
Vahlkampfia spp.	JITS	Gtcttcgtaggtgaacctgc	18S rRNA gene	About 500	**De Jonckheere & Brown, 2005**
	JITS	Ccgcttactgatatgcttaa			
Vannella spp.	NA1	Gctccaatagcgtatattaa	18S rRNA gene	800	**Lasjerdi *et al*., 2011**
	NA2	Agaaagagctatcaatctgt			

Table 2: PCR thermal profile for tested free-living amoebae

Protozoa	Pre-denaturation	Thermal cycles	Final extension	Reference
Acanthamoeba spp.	95^{O}C for 3min	35 cycles each at 95^{O}C for 30sec 63^{O}C for 30sec 72^{O}C for 90sec	72°C for 10min	**Qvarnstrom *et al*., 2006**
Naegleria spp.	95^{O}C for 10min	40 cycles each at 95^{O}C for 30sec 58 ^{O}C for 30sec 72^{O}C for 30sec	72°C for 10min.	**Schild *et al*., 2007**
Hartmannella vermiforms	95^{O}C for 3min	35 cycles each at 94^{O}C for 30sec 63^{O}C for 30sec 72^{O}C for 30sec	72 °C for 10min	**Kuiper *et al*., 2006**
Vahkamphia spp.	95^{O}C for 3min	35 cycles each at 95^{O}C for 30sec 55^{O}C for 30sec 72^{O}C for 90sec	72°C for 10min	**De Jonckheere and Brown, 2005**
Vannella spp.	95^{O}C for 10min	40 cycles each at 95^{O}C for 30sec 57 ^{O}C for 30sec 72^{O}C for 30sec	72°C for 10min.	**Lasjerdi *et al*., 2011**

RESULTS AND DISCUSSION

Ensuring the microbiological safety of drinking water is of paramount importance. So, source water quality should be routinely characterized. Monitoring of source water for protozoa can be targeted by using information about sources of fecal contamination from a sanitary survey, together with historical data on rainfall, river flow and turbidity, to help to identify the conditions that are likely leading to peak

events (Federal-Provincial-Territorial Committee on Health and the Environment, 2012).

In the present study, pathogenic free-living amoebae were detected in 87.5% and 91.7% of raw water samples from the examined conventional and compact drinking water treatment plants, respectively. Consequently, FLAs were detected in 20.8% and 45.8% of treated (finished) water samples from conventional and compact DWTPs, respectively (Table 3). Other researches, conducted in Minofeya governorate, Egypt, declared that all examined water samples from the inlet (raw surface water) of Shebeen Elkom DWTP had pathogenic free-living amoebae (Zard, 2017; Al-Herrawy *et al.,* 2017). The predominance of free-living amoebae in various water types are resistant to extreme conditions of temperature, pH, and exposure to various chemicals (Thomas *et al.,* 2008). Unlike true parasites, pathogenic FLAs can complete their life cycles in the environment without entering a human or animal host. Some of FLAs are pathogenic for humans (Martinez and Visvesvara, 1997).

In another work in Fayoum governorate, Egypt, the occurrence of potentially pathogenic free-living amoebae ranged between 58.3% and 91.7% in different surface water inlets of 4 DWTPs (Al-Herrawy *et al*., 2015). These differences reflected the difference of raw water quality in different sites of the same country.

Table 3: Total occurrence of potentially pathogenic FLAs in the examined DWTPs by culture and PCR.

	Conventional DWTP				**Compact units**			
	Raw		**Finished**		**Raw**		**Finished**	
	Culture	**PCR**	**Culture**	**PCR**	**Culture**	**PCR**	**Culture**	**PCR**
Examined samples	24	24	24	24	24	24	24	24
Positive samples	21	18	5	4	22	21	11	9
%	87.5	75.0	20.8	16.7	91.7	87.5	45.8	37.5

Concerning the removal of free-living amoebae from drinking water, it was shown that conventional DWTP could get rid of 76.2% of FLAs present in the raw untreated water, while compact units removed only 50% of these organisms (Table 4). Also, the presence of free-living amoebae in the finished water (completely treated drinking water) was an indication that there were some defects in the application and performance of drinking water treatment steps.

Table 4: Removal of pathogenic free-living amoebae in the examined DWTPs.

	Pathogenic free-living amoebae			
	Conventional DWTP		**Compact units**	
	Raw	Finished	Raw	Finished
+ve samples	21	5	22	11
Removed		16		11
Removal %		76.2%		50%

The presence of cyst stage (hard, dormant and persistent to harsh environmental conditions) in most species of FLAs (except *Vannella*) facilitated their escape from drinking water treatment steps. Also, the ability of FLAs to reproduce in the environment, without the need for a host, enabled these organisms to reproduce and increase in numbers wherever the suitable environmental conditions were present (Aksozek *et al,* 2002). Cysts have been known to survive in vitro for greater or equal to 20 years under adequate humidity and suitable temperature (Mazur *et al,* 1995). With the return of optimal and favorable conditions for growth, especially the presence of food, cysts germinate to give rise to trophic forms.

Statistically, the conventional drinking water treatment plant had a strong significant effect for removal of free-living amoebae (P-Value = 0.000) from the inlet raw water (Table 5). Also, the compact units for drinking water treatment had a significant effect for removal of free-living amoebae (P-value = 0.001). The conventional DWTP was more efficient than compact units for removal of FLAs (Table 6).

Table 5: Paired T test for FLAs in raw versus finished water of conventional DWTP.

	N	Mean	St Dev	SE Mean
Raw conventional DWTP	24	0.875000	0.337832	0.068960
Finished conventional DWTP	24	0.208333	0.414851	0.084681
Difference	24	0.666667	0.564660	0.115261

95% CI for mean difference: (0.428232; 0.905102)
T-Test of mean difference = 0 (vs not = 0): T-Value = 5.78 P-Value = 0.00

Table 6: Paired T test for FLAs in raw versus finished water of compact units

	N	Mean	St Dev	SE Mean
Raw compact units	24	0.916667	0.282330	0.057630
Finished compact units	24	0.458333	0.508977	0.103895
Difference	24	0.458333	0.588230	0.120072

95% CI for mean difference: (0.209946; 0.706721)
T-Test of mean difference = 0 (vs not = 0): T-Value = 3.82 P-Value = 0.001

In comparison between inlets of the two examined drinking water treatment plants, the obtained data declared that the inlet of compact units was more contaminated with parasitic protozoa and pathogenic FLAs than that of conventional DWTP (Table 3). At the same time, statistical analysis revealed that there was no significant difference (P-Value = 0.313) between the prevalence of FLAs in raw water collected from the inlets of both conventional DWTP and compact units **(**Table 7).

Table 7: Two-Sample T-Test and CI: FLAs in raw water of conventional DWTP versus raw water of compact units

	N	Mean	St Dev	SE Mean
Raw conventional DWTP	24	0.833	0.381	0.078
Raw compact units	24	0.708	0.464	0.095

Difference = mu (Raw Conventional) - mu (Raw Compact) Estimate for difference: -0.125000 95% CI for difference: (-0.372005; 0.122005), T-Test of difference = 0 (vs not =): T-Value = -1.02 P-Value=0.313

The present work showed that there was a pronounced difference between the presence of pathogenic FLAs in produced drinking water from the two examined DWTPs. The finished water produced by conventional DWTP was less contaminated with pathogenic FLAs than finished water produced by compact units (Table 3). Consequently, statistical analysis proved that the obtained laboratory results agreed with that obtained by statistical analysis. Statistically, there was no significant difference (P-Value = 0.780) between the prevalence of FLA in the finished water obtained from the two different DWTP (Table 8).

With respect to genera of FLAs isolated in the present work, 5 genera (*Acanthamoeba, Vahlkampfia, Hartmannella*, *Naegleria* and *Vannella*) were isolated from raw water samples, while only 2 genera (*Acanthamoeba* and *Hartmannella*) can persist treatment and escape to fully treated drinking water. In general, positive samples for FLAs in compact units exceeded those for FLAs in conventional DWTP.

Table 8: wo-Sample T-Test and CI: FLAs in finished water of conventional DWTP versus finished water of compact units

	N	Mean	St Dev	SE Mean
Finished conventional DWTP	13	0.231	0.439	0.12
Finished compact units	11	0.182	0.405	0.12

Difference = mu (0) - mu (1)
Estimate for difference: 0.048951
95% CI for difference: (-0.310782; 0.408684)
T-Test of difference = 0 (vs not =): T-Value = 0.28 P-Value = 0.780 DF = 22
Both use Pooled St Dev = 0.4234.

Members of genus *Acanthamoeba* were the most predominant FLAs in examined samples, followed by *Hartmannella, Vahlkampfia, Naegleria* and lastly *Vannella* (Table 9 and Figs. 1 & 2). These findings were nearly in accordance with those presented by Al-Herrawy and Gad (2017) in the examined Fayoum drinking water treatment plants and by Al-Herrawy *et al.* (2016) in swimming pools and sea water in Egypt. The basis for novel anti-infection therapies to unravel the host-pathogen interactions has begun. Last but not least, amoebae as host cells are not constricted to bacteria or fungi. They can also be used to study the interaction with viruses such as the giant viruses from the ecological, evolutionary and medical point of view (Thewes *et al*., 2019).

Table 9: Genera of pathogenic FLAs in the two examined drinking water treatment facilities by culture and PCR

		Conventional DWTP				**Compact units**			
		Raw		Finished		Raw		Finished	
		Culture	PCR	Culture	PCR	Culture	PCR	Culture	PCR
Examined samples		24		24		24		24	
Positive samples +ve %	Aca	15(62.5)	13(54.2)	5(20.8)	4(16.7)	18(75.0)	16(66.7)	9(37.5)	8(33.3)
	Har	6 (25.0)	6(25.0)	-	-	9 (37.5)	8(33.3)	1 (4.2)	1(4.2)
	Nae	3 (12.5)	3(12.5)	-	-	4 (16.7)	3(12.5)	-	-
	Vah	2 (8.3)	0	-	-	7 (29.2)	0	-	-
	Van	1 (4.2)	0	-	-	1 (4.2)	0	-	-

Aca=*Acanthamoeba* Har=*Hartmannella* Nae=*Naegleria* Vah=*Vahlkamfia* Van=*Vannella*

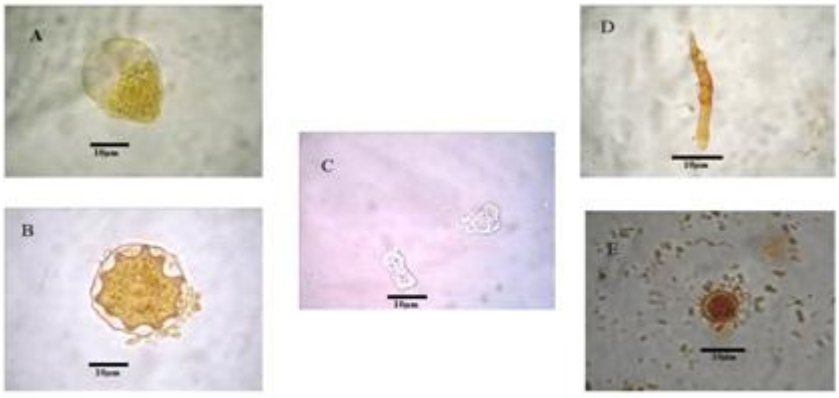

Figure 1. Photomicrographs for detected pathogenic FLAs. A: *Vannella* trophozoite; B: *Acanthamoeba* cyst; C: *Naegleria* trophozoite; D: *Hartmannella* trophozoite; E: *Vahlkampfia* cyst.

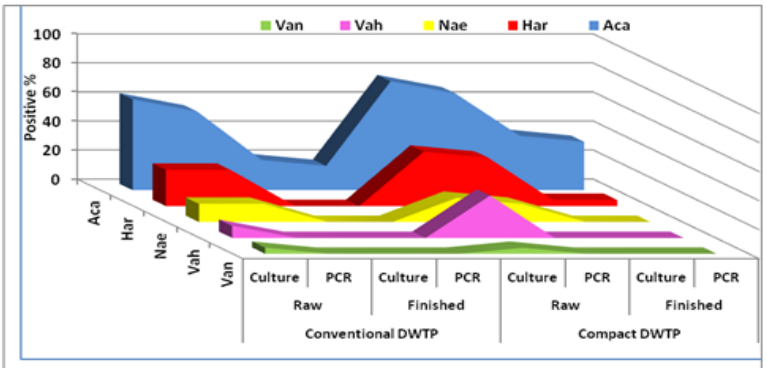

Figure 2. Occurrence of genera of pathogenic FLAs in the examined DWTPs by culture and PCR

The present results showed that members of genus *Acanthamoeba* were the predominant FLAs in examined raw and finished water from both systems of drinking water treatment facilities (conventional and compact). Moreover, genus *Acanthamoeba* showed higher occurrence in compact units than in conventional DWTP. The same behavior occurred with *Hartmanella*, *Vahlkampfia* and *Naegleria*, while *Vannella* exhibited equal distribution in the 2 different treatment systems (Table 9 and Figure 2). These results were in accordance with that obtained by Zard (2017) in Minofeya governorate and Zaghlol (2015) in Fayoum governorate. Recognition of *Acanthamoeba* spp. at the genus level was based on distinguishing features of trophozoites and cysts, especially the double-walled cyst shape that was a unique to the genus. *Acanthamoeba* species have been classified into three different morphological groups (Pussard and Pons, 1977).

Genera of FLAs other than *Acanthamoeba* were detected in lower incidences. In other words, all the detected genera of FLAs, except *Acanthamoeba*, in raw water samples of conventional DWTP disappeared completely from finished (tap) water. Concerning compact units for drinking water treatment, members of genera *Acanthamoeba* and *Hartmannella* could persist treatment and escaped to finished water (Table 9 and Figure 2). In our opinion, the double and hard cyst wall of *Acanthamoeba* and *Hartmannella* facilitated the escape of these amoebae from the killing action of chlorine used for disinfection of drinking water. Moreover, free-living and parasitic protozoa have been reported as causative agents of illness and after their exposure to higher concentrations of disinfectants, protozoa can survive longer than bacteria and viruses (Bonadonna *et al.*, 2013).

Waterborne parasites such as potentially pathogenic FLAs, that may cause severe health effects in humans and animals, have not been widely studied in developing countries. However, they have been studied more in developed countries and are known to be associated with severe infections in humans. In sub-Saharan Africa, due to lack of information, more studies are needed to establish the health importance of FLAs (Centers for Disease Control and Prevention, 2015). Several bacteria-fungi interactions have been reported, but mycorrhizal fungi mainly appeared as fungal organisms involved. Selective proliferation of bacteria on the proximity of mycelia of different fungi and introduced the concept of fungiphillic bacteria (Bacteria adapted to the exploitation of hyphal exudates as a carbon source (Bystransky *et al*., 2019).

CONCLUSION

The intakes of the examined drinking water treatment facilities harbored potentially pathogenic FLAs. The presence of potentially pathogenic free-living

amoebae in raw water and finished water should be considered a potential health threat. Regular examination of drinking water for the presence of FLAs must be performed as a routine test to prevent incidence of waterborne prootozoan outbreaks. The conventional DWTP was more efficient than compact units for the removal of potentially pathogenic FLAs. Culture methods demonstrate only the viable pathogenic FLAs in water, while the traditional PCR technique is responsible for the detection of genomes, whether they are living or dead. Better surveillance and management strategies are needed to assess the risk of waterborne transmission of these pathogens.

RECOMMENDATION

Further monitoring studies are required to understand the presence and circulation of FLAs in the Egyptian environment, particularly for *Acanthamoeba* species, to prevent future public infections.

ACKNOWLEDGEMENTS

This work was financially supported by National Research Centre, In-house project number 11060101.

Conflict of Interests

The authors declare that there is no conflict of interests.

REFERENCES

Aksozek, A.; McClellan, K.; Howard, K.; Niederkorn, J.V. and Alizadeh, H. (2002). Resistance of *Acanthamoeba castellanii* cysts to physical, chemical, and radiological conditions. J. Parasitol., 88: 621-623.

Al-Herrawy, A.Z.; El-Mohandes, M.A.; Al-Gendy, A.A.R.; Ahmed, W.F.A. and Abdelaziz, Y.A. (2017). Existence of free-living amoebae in operationally different drinking water treatment plants having different water sources in Minofeya governorate, Egypt. Egypt. J. Environ. Res., 8: 1-17.

Al-Herrawy, A.Z. and Gad, M.A. (2017). Assessment of two different drinking water treatment plants for the removal of free-living amoebae, Egypt. Iran. J. Parasitol., 12: 413-422.

Al-Herrawy, A.Z.; Gad, M.A.; Abd El-Aziz, A.; Abou El-Nour, M.F.; Shaldoum, F.M. and Salahuldeen, A. (2016). Morphological and molecular detection of potentially pathogenic free-living amoebae in swimming pool samples. Egypt. J. Environ. Res., 5: 1-13.

Al-Herrawy, A.Z.; Mohamed, S.H.; Mohamed, A.H. and Zaghloul, N.M. (2015). Surveillance of potentially pathogenic free-living amoebae through drinking water treatment processes in Fayoum governorate, Egypt. Intern. J. Environ., 4: 98-107.

Amir, S. (2009). *Acanthamoeba castellanii* as a host and model to study bacterial virulence. Thesis, Karolinska Universitet, Stockholm, Sweden.

Anjum, U.; Magnet, A.; Acosta, L.; Toledo, R. and Fernandez, A. (2019). Are human pathogenic free-living amoebae present in Liesester city,s environment (UK)?. XXI Congreso SOCEPA, Pontevedra, 3-5 Julio 2019, Pagina 166-344.

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

Bonadonna, L.; Briancesco, R.; Paradiso, R. and Semproni, M. (2013). Free-living amoebae and enteric protozoa in swimming pool. In: Ferretti, E., Fantuzzi, G., Spica, V.R., Caroli, S. and Bonadonna, L. (eds) the 5th international conference swimming pool and spa. Instituto Superior di Sanita and Universita di Roma Faro Italico Rome.

Bystransky, L.; Hujslova, M.; Hrselova, H.; Rezacova, V.; Nemcova, L.; Simsova, J.; Gryndlerova, H.; Kofronova, O.; Benada, O. and Gryndler, M. (2019). Observations on

two microbial life strategies in soil: Planktonic and biofilm-forming microorganisms are separable. Soil Biology and Biochemistry 136: 1-11.

Centers for Disease Control and Prevention, USA (2015). Domestic water, sanitation, and hygiene epidemiology. Atlanta, USA: Centers for Disease Control and Prevention, United States of America.

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: 507-528.

De Jonckheere, J.F. and Brown, S. (2005). The identification of vahlkampfiid amoebae by ITS sequencing. Protist., 156: 89–96.

Dey, R.; Hoffman, P.S. and Glomski, I.J. (2012). Germination and amplification of anthrax spores by soil-dwelling amoebas. Appl Environ Microbiol., 78(22): 8075–81.

Federal-Provincial-Territorial Committee on Health and the Environment (2012). Guidelines for Canadian Drinking Water Quality. Guideline technical document. Enteric protozoa: *Giardia* and *Cryptosporidium*. Federal-provincial-territorial committee on health and the environment, Health Canada Ottawa, Ontario.

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

Hara, T.; Yagita, K. and Sugita, Y. (2019). Pathogenic free-living amoebic encephalitis in Japan. Neuropathology, 39(4): 251-258.

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.

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

Kuiper, M.W.; Valster, R.M.; Wullings, B.A.; Boonstra, H.; Smidt, H. and van der Kooij, D. (2006). Quantitative detection of the free-living amoeba *Hartmannella vermiformis* in surface water by using real-time PCR. Appl. Environ. Microbiol., 72: 5750–5756.

Lasjerdi, Z.; Niyyati, M.; Haghighi, A.; Shahabi, S.; Biderouni, F.T.; Taghipour, N.; Eftekhar, M. and Nazamalhosseini Mojarad, E. (2011). Potentially pathogenic free-living amoebae isolated from hospital wards with immuno-deficient patients in Tehran, Iran. Parasitol. Res., 109: 575–580.

Mazur, T.; Hadas, E. and Hvanieka, I. (1995). The duration of the cyst stage and the viability and virulence of *Acanthamoeba* isolates. Trop. Med. Parasitol., 46: 106-108.

Page, F.C. (1988). A new key to freshwater and soil Gymnamoebae. Freshwater Biol. Ass., Ambleside.

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.

Pussard, M. and Pons, R. (1977). Morphologie de la paroikystique et taxonomie du genre *Acanthamoeba*. Protistologica, 13: 557-598.

Qvarnstrom, Y.; Visvesvara, G.S.; Sriram, R. and Da Silva, A.J. (2006). Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. J. Clinic. Microbiol., 44: 3589–3595.

Schild, M.; Gianinazzi, C.; Gottstein, B. and Müller, N. (2007). PCR-based diagnosis of *Naegleria* sp. infection in formalin-fixed and paraffin-embedded brain sections. J. Clinic. Microbiol., 45: 564–567.

Thewes, S.; Soldati, T. and Eichinger, L. (2019). Amoebae as host models to study the interaction with pathogens. Frontiers Cell. Infect. Microbiol., 9: 1-3.

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

Visvesvara, G.S.; Moura, H. and Schuster, F.L. (2007). Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol. Med. Microbiol., 50(1): 1–26.

WHO (2004). Water Treatment and Pathogen Control. IWA Publishing, Alliance House, London. UK.

WHO (2011). Guidelines for drinking-water quality, 4th edition. 1211 Geneva, Switzerland.www.who.int/water_sanitation_health/publications/2011/dwq_chapters/en.

Zahglol, N.R. (2015). Occurrence of free-living amoebae in drinking water. M.Sc. thesis, Faculty of Science, Ain Shams University, Cairo, Egypt.

Zard, Y.A. (2017). Control of pathogenic free-living amoeba in different water resources at El-Minofeya governorate. M.Sc. thesis in Environmental Agriculture Sciences, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

ARABIC SUMMARY

الكشف عن وإزالة الأميبات حرة المعيشة الممرضة في نوعين مختلفين من محطات تنقية مياه الشرب عن طريق الزرع وتفاعل البلمرة المتسلسل

أحمد زكريا الهراوي[١*] ، محمد علي معروف[١] ، نورا ربيع زغلول[٢]، محمود عفو جاد[١]

١- معمل الطفيليات البيئي - قسم بحوث تلوث المياه - المركز القومي للبحوث - جيزه - مصر

٢- المعمل المركزي - شركة مياه الشرب والصرف الصحي بالفيوم - العزب الجديدة - الفيوم - مصر

تعد محطات تنقية مياه الشرب التقليدية (CDWTPs) والوحدات المدمجة (CUs) أهم نوعين من محطات تنقية مياه الشرب التي تستخدم المياه العذبة كمصدر لمياه الشرب في مصر ، وحيث أن المعايير القياسية المصرية لمياه الشرب تمنع وجود أي نوع من الكائنات الحية في مياه الشرب المنتجة للاستخدام البشري. لذا فإن هدف الدراسة الحالية هو المقارنة بين كفاءة نوعين مختلفين من طرق تنقية مياه الشرب المختلفة (CDWTP و CU) في إزالة الأميبات حرة المعيشة المسببة للأمراض.

في هذه الدراسة ، تم جمع عينات المياه الخام والتشطيب بشكل منفصل من CDWTP و CU في محافظة الجيزة ، مصر. تم تركيز العينات بشكل منفصل من خلال مرشحات غشاء النيتروسليلوز (حجم المسام ٠.٤٥ميكرون). تم الكشف عن وجود الأميبات الحرة الممرضة في المركزات عن طريق الزراعة و PCR.

باستخدام المجهر ، تمت التعرف علي خمسة أجناس من الأميبات حرة المعيشة (*Acanthamoeba* و *Vahlkampfia* و *Hartmannella* و *Naegleria* و *Vannella*) من عينات المياه الداخلة لكل نوع من نظامي تنقية مياه الشرب. أظهرت البيانات التي تم الحصول عليها أن ٨٧.٥ ٪ و ٩١.٧ ٪ من المياه الداخلة إلي كل نظام (CDWTP و CU)، على التوالي تحتوي على أميبات حرة المعيشة ممرضة، كذلك تم عزل هذه الأميبات أيضا من ٢٠.٨ ٪ و ٤٥.٨ ٪ من عينات مياه الشرب الخارجة من CDWTP و CU ، على التوالي.

تم التعرف علي *Acanthamoeba* في عينات مياه الشرب النهائية لكل من CDWTP و CU ، في حين تم عزل *Hartmannella* من مياه الشرب النهائية الناتجة من CU فقط. وصلت نسبة إزالة الأميبات حرة المعيشة الممرضة إلى ٧٦.٢ ٪ في CDWTP ، في حين وصلت إلى ٥٠ ٪ فقط في CU. باستخدام تفاعل البلمرة المتسلسل تم التعرف علي ثلاثة أجناس فقط من الأميبات هي *Acanthamoeba* و *Hartmannella* و *Naegleria* بنسبة حدوث أقل من تلك التي كشفت عنها طريقة الزرع والفحص الميكروسكوبي.

في الختام ، تحتوي عينات المياه الداخلة لكل من CDWTP و CU على أميبات حرة المعيشة مسببة للأمراض. وعلى الرغم من أن CDWTP كان أكثر فعالية من CU في إزالة هذه الأميبات ، إلا أنه لا يزال من الممكن اكتشاف بعض هذه الكائنات في المياه النهائية المعدة للشرب وبالتالي تسبب مخاطر صحية على المستهلكين.