Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(3): 61 – 73 (2020)
www.ejabf.journals.ekb.eg

Distribution of potentially pathogenic *Acanthamoeba* isolates in the environment of Helwan University, Egypt

Heba Koteit[1]; Shehata E. Elowa[1]; Ahmad Z. Al-Herrawy[2]*

1- Zoology and Entomology Department, Faculty of Science, Helwan University, Egypt
2- Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt
*Corresponding Author: alherrawy@gmail.com

ARTICLE INFO
Article History:
Received: Jan.3, 2020
Accepted: April 28, 2020
Online: May 2020

Keywords:
Acanthamoeba,
Environment,
Helwan University,
Egypt

ABSTRACT

Acanthamoeba species are free-living amoebae having worldwide distribution. These amoebae can cause granulomatous amoebic encephalitis and amoebic keratitis in humans. They can produce proteases that are considered virulence factors. *Acanthamoeba* can also harbor pathogenic bacteria, fungi, and viruses.

The objective of this study is to evaluate the presence of *Acanthamoeba* in the environment of Helwan University, Egypt.

Six types of samples (tap water, irrigation water, wastewater, swabs from surfaces, soil, and air) were collected, processed, and cultured on non-nutrient agar medium. Positive plates for *Acanthamoeba* were subcultured, purified and amoebae were identified morphologically and confirmed by PCR using *Acanthamoeba* genus-specific primers. Obtained results declared that members of genus *Acanthamoeba* were detected in 91.7, 83.3, 54.2, 45.8, 12.5 and 12.5% of irrigation water, soil, swabs, wastewater, tap water, and air samples, respectively. The morphologically identified *Acanthamoeba* species proved to be related to genus *Acanthamoeba* when tested by PCR. Statistically, the sampling source had a strong significant correlation with the prevalence of *Acanthamoeba*. The highest appearance of *Acanthamoeba* was recorded in the spring season for samples from irrigation water, soil, and swabs from surfaces.

In conclusion, the high prevalence of *Acanthamoeba* species in irrigation water and soil exert public health hazards to students and workers in Helwan University.

INTRODUCTION

Acanthamoeba was first isolated in 1913 by Puschkarew as amoeba from the dust and named *Amoeba polyphagus*. Later in **1930**, Castellani isolated an amoeba that occurred as a contaminant in a culture of the fungus *Cryptococcus pararoseus* **(Castellani, 1930)**. From that time until now, *Acanthamoeba* species show up their ability to survive in diverse environments. Consequently, they have been isolated from these environments

Indexed in Scopus ELSEVIER DOAJ IUCAT

and even from the atmosphere. In addition, *Acanthamoeba* have been recovered from hospitals, dialysis units, eye wash stations, corneal biopsies, skin lesions, human nasal cavities, pharyngeal swabs, lungs tissues, cerebrospinal fluid (CSF) and brain necropsies **(Khan, 2003; Marciano-Cabral and Cabral, 2003; Schuster and Visvesvara, 2004)**.

Acanthamoeba trophozoite possesses a large number of mitochondria (**Burger *et al*., 1995**). *Acanthamoeba* trophozoite moves a relatively fast, with a locomotion rate of approximately 0.8 μm /second. The movement involves the formation of a hyaline pseudopodium called acanthopodium **(Preston *et al*., 2001).**

Under harsh conditions, the trophozoites differentiate into a non-dividing, double-walled resistant cyst form. Cyst walls contain cellulose (not present in the trophozoite stage) that accounts for 10% of the total dry weight of the cyst although cyst wall composition varies between isolates belonging to different species and genotypes **(Derda *et al*., 2009; Dudley *et al*., 2009)**. The most abundant *A. castellanii* cyst wall proteins are three sets of lectins, which have carbohydrate-binding modules **(Magistrado-Coxen *et al*., 2019).**

Acanthamoeba, a free-living amoeba, is an opportunistic pathogen of humans and other animals including gorills, monkeys, dogs, ovines, horses and kangaroos, as well as birds, reptiles, amphibians, and fishes **(Martinez and Visvesvara, 1997; Dykova *et al*., 1999).**

Acanthamoeba is the most common cause of illness, usually infecting the eyes and sometimes causing a sight-threatening keratitis **(Yoder *et al.*, 2010)**. *Acanthamoeba* spp. can also cause a highly fatal CNS infection known as granulomatous amoebic encephalitis (GAE), in addition to infections of the lungs and skin **(Visvesvara *et al.*, 2007; Visvesvara, 2010).**

Acanthamoeba cysts can withstand desiccation for more than 20 years. It is therefore necessary to continuously monitor isolates of *Acanthamoeba* for their resistance to environmental pollutions **(Sriram *et al.*, 2008).** So, the aim of the present work is to remind the decision-makers about the presence of potentially pathogenic *Acanthamoeba* species in the environment of Helwan University and announcing their hazards on the students.

MATERIALS AND METHODS

Samples and sampling sites

A total of 144 samples were collected from Helwan University environment during one year period from March 2017 to February 2018. Different types of environmental samples were collected (Tap water, irrigation water, wastewater, soil, swabs from surfaces and air samples). Samples were regularly collected two times per month during the study period. Collection of samples was performed following to **Health Protection Agency (2004)** and **American Public Health Association (2017)** as follows:

- Water samples (from tap, irrigation and wastewaters) were separately collected (1L volume each) in clean, dry and autoclavable polypropylene containers.

- Soil samples (about 100g each) were separately collected from the gardens of Helwan University in sterile autoclavable polypropylene plastic beakers that were then wrapped with parafilm.
- Swabs were separately collected from bench surfaces of laboratory number 3 of Zoology and Entomology Department, Faculty of Science by sterile cotton swabs stored in 10ml sterilized Page's saline **(Page, 1988)**.
- Air samples were collected by leaving uncovered non–nutrient (NN) agar plates, socked with heat-killed *Escherichia coli* suspension, in direct contact with air at different areas outside the buildings. The plates were left opened for 2hr then covered with their lid, sealed with parafilm and immediately transported to the laboratory for incubation.

After collection, all samples were transported at ambient temperature in an ice box to Environmental Parasitology Laboratory, Water Pollution Research Department, National Research Centre, Dokki, Giza where they were processed at the same day of collection**.**

Processing and cultivation of samples

About 100g from every soil sample were separately added to 1L autoclaved Page's saline with vigorous shaking for 10min and then left to settle for 5min. The supernatant was siphoned and treated as a water sample.

Water samples (whether tap water, irrigation water, wastewater and supernatant of soil samples) were separately filtered through a nitrocellulose membrane (0.45µm pore size and 47mm in diameter) using a stainless steel filter holder connected with a suction pump. Filtration was stopped just before drying of the membrane **(Health Protection Agency, 2004; American Public Health Association, 2017).** After filtration process, the membrane was inverted face to face on the surface of NN agar plate seeded with heat-killed *Escherichia coli*.

Swab samples in Page's saline were centrifuged at 1500xg for 10min. The last 1ml of centrifuged Page's saline of each swab sample was spread on the surface of NN agar plate seeded with heat-killed *E. coli* bacteria.

All the inoculated plates, in addition to air samples, were wrapped with parafilm and incubated at 30^{o}C for one week **(Page, 1988; American Public Health Association, 2017).** Incubated plates were daily examined by the inverted microscope (Olympus CXK 41, Japan) for the presence of any amoebic growth.

Morphological identification of isolated FLAs

The cloned amoebae (both trophozoites and cysts) on plates were morphologically examined for the presence of FLAs and identification of those belonging to *Acanthamoeba* according to the key described by Page **(Pussard and Pons, 1977; Page, 1988).** Amoebae, suspected to be *Acanthamoeba*, were sub-cultured to isolate and purify grown amoebae for further investigations **(Al-Herrawy, 1992).**

Molecular confirmation of the isolated *Acanthamoeba* by polymerase chain reaction (PCR) (Schroeder *et al*., 2001).

A simple PCR technique was used, consisting of DNA extraction and amplification followed by agarose gel electrophoresis**.**

Acanthamoeba DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) following the manufacturer instructions. PCR was done to amplify a restricted fragment of DNA through generic primers (JDP1 and JDP2) for identification of *Acanthamoeba* species (Table 1).

Each PCR reaction was carried out in a final volume of 50 µl (25µl master mix "Promega, USA", 3µl template DNA, 2µl forward and reverse primers and 20µl diethylpyrocarbonate "DEPC-treated water"). The amplification program included an initial denaturation at 95°C for 5min, followed by 35 cycles; each consisted of denaturation at 94°C for 30sec., annealing at 55°C for 40sec and extension at 72°C for 40sec. The program included a final extension step at 72°C for 10min to generate amplification fragments from 423-551bp **(Schroeder *et al.,* 2001).** The obtained PCR products were visualized and photographed using agarose gel electrophoresis and documentation system**.**

Table 1. Sequence of a primer pair for detection of genus *Acanthamoeba.*

Organism	Primer direction	Primer sequence (5`- 3`)	Reference
***Acanthamoeba* spp.**	**Forward**	**GGCCCAGATCGTTTACCGTGAA**	**Schroeder *et al.* (2001)**
	Reverse	**JTCTCACAAGCTGCTAGGGAGTCA**	

Statistical analysis

The obtained data were statistically analyzed using GraphPad Prism version 7.0 (USA) software. The critical *P*-value for the test was set at <0.05.

RESULTS AND DISCUSSION

Members of genus *Acanthamoeba* exist in nature either as a trophic amoeba feeding on bacteria present in soil and water, or as a non-feeding dormant cyst. The trophic form of *Acanthamoeba* is characterized by the presence of thorn-like pseudopodia called acanthopodia and there is no flagellate form. The cyst form is characterized by a double-layered cyst wall having a varying number of pores **(Pussard and Pons, 1977**).

Acanthamoeba species were isolated from all the collected environmental samples from Helwan University. Morphologically, the trophozoites of different *Acanthamoeba* species were nearly similar. They have finger-like locomotive projections arising from the cytoplasm. However, these trophozoites varied in length from 20 to 45µm and ranged from 15 to 30µm in width. The outline of an amoeba was often irregular but it was generally longer than broad. A single vesiculate nucleus was seen in the anterior half of endoplasmic region. The nucleus measured 4 – 8µm in diameter and had a

characteristically large centrally located dense nucleolus surrounded by a clear halo and thin nuclear membrane (Figure 1A). The cyst form of *Acanthamoeba* species was characterized by the presence of a double cyst wall (ectocyst and endocyst). An *Acanthamoeba* cyst had a smooth or wrinkled outer wall (ectocyst) and a stellate, polygonal, star– like or even inner wall (endocyst) and measured 12 to 25µm in diameter. There were plugged pores scattered on surface of the cyst wall; these pores were covered by opercula. Also, *Acanthamoeba* cysts had different shapes which were species specific (Figure 1B). All the morphologically detected *Acanthamoeba* proved to be belonging to genus *Acanthamoeba* when tested by PCR using a genus-specific primer pair (Figure 2). Other workers used riboprinting (RFLP analysis of the 18S small subunit ribosomal RNA (srRNA) gene) for the classification of *Acanthamoeba* species at the subgenus level **(Chung *et al*, 1998; Kong and Chung, 2002).**

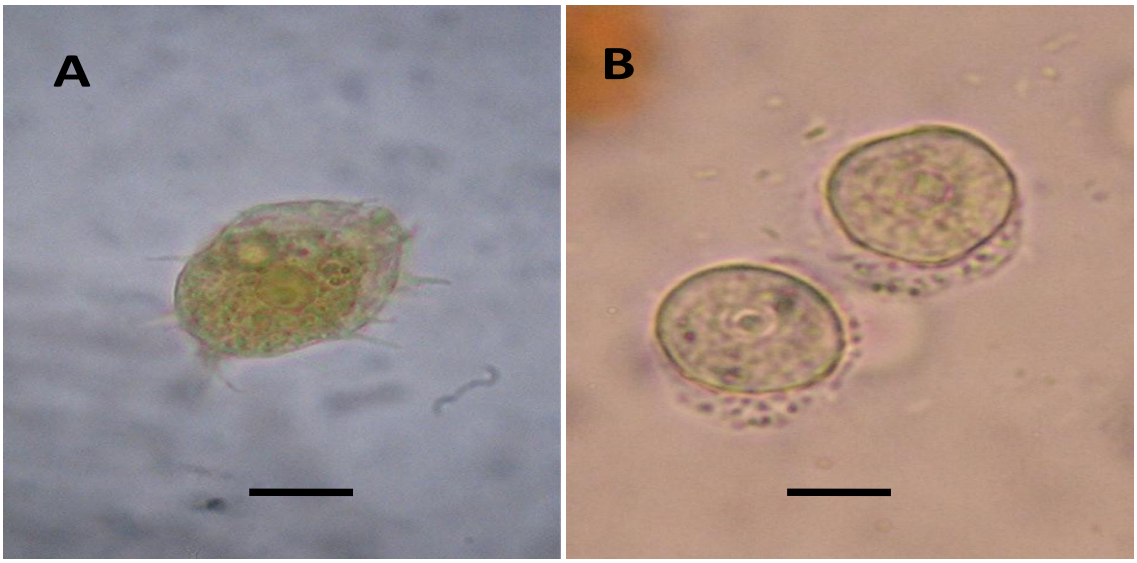

Figure 1. Photomicrograph for *Acanthamoeba* species .
A) Trophozoite B) Cyst Bar = 10µm

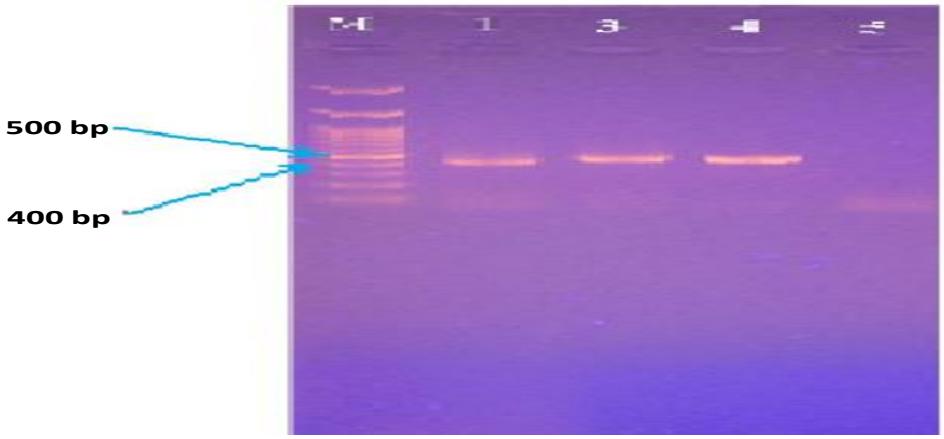

Figure 2. Agarose gel electrophorisis for PCR amplified product of DNA from *Acanthamoeba* spp. M: Marker; Lane 1: Control positive; Lanes 3 and 4: Positive samples; Lane 5: Control negative.

Acanthamoeba species were isolated, in the present investigation, from all environmental samples of Helwan University. Examination of 144 environmental samples collected from Helwan University revealed that the highest percentage of *Acanthamoeba* (91.2%) was recorded from irrigation water samples, soil (83.3%), swabs samples from surfaces (54.2 %), domestic wastewater (50%), and lastly tap water and air samples with a similar occurrence (13%) for each (Table 2 and Figure 3).

In a previous study conducted on tap water from five governorates in Egypt, 26.6% out of 180 tap water samples were positive for *Acanthamoeba* species. They also found that Faiyum governorate was the highest site for occurrence of *Acanthamoeba* in tap water 36.1% (13/36), followed by Helwan 27.8% (10/36) and Cairo was the lowest site for occurrence of *Acanthamoeba* 19.4% (7/36) **(Gad *et al.*, 2019).** Other several studies, conducted previously in Egypt, recorded that 80%, 58.6%, 56.3%, 31.4%, 67.7% and 29.2% of drinking water samples, collected from Beni-Suef governorate, Nile Delta governorates, Giza governorate, Cairo governorate and Faiyum governorate, respectively, were positive for *Acanthamoeba* species **(Gad and Al-Herrawy, 2016; Morsy *et al.*, 2016; Tawfeek *et al.*, 2016; Sakran *et al.*, 2017; Al-Herrawy *et al.*, 2017; Abd El Wahab *et al.*, 2018).** Globally**,** *Acanthamoeba* spp. have been documented in tap water in Korea (5.8%) Nicaragua (19%), Turkey (4.4% and 26.8%) and Philippines (9.1%) **(Jeong and Yu, 2005; Leiva *et al.*, 2008; Coşkun *et al.*, 2013; Onichandran *et al.*, 2014).** In our opinion, there are big differences in detection rates of *Acanthamoeba* in different sites and countries due to the difference in geographic areas, the quality of raw water sources or additional treatment technologies facilities in each country.

Statistical analysis of the obtained data revealed that the sampling source and types of samples had a strong significant correlation ($P<0.0001$ and $R^2=0.3784$) with the prevalence of *Acanthamoeba* in the environment of Helwan University (Table 3).

Table 2. Distribution of genus *Acanthamoeba* in environmental samples from Helwan University

Season	Examined samples for each site	*Acanthamoeba* positive samples					
		Irrigation water	Soil	Swabs from surface	Waste Water	Tap water	Air
Winter	6	5	4	1	3	1	0
Spring	6	6	6	6	3	1	0
Summer	6	6	5	3	2	0	3
Autumn	6	5	4	3	3	1	0
Total	24	22	20	13	11	3	3

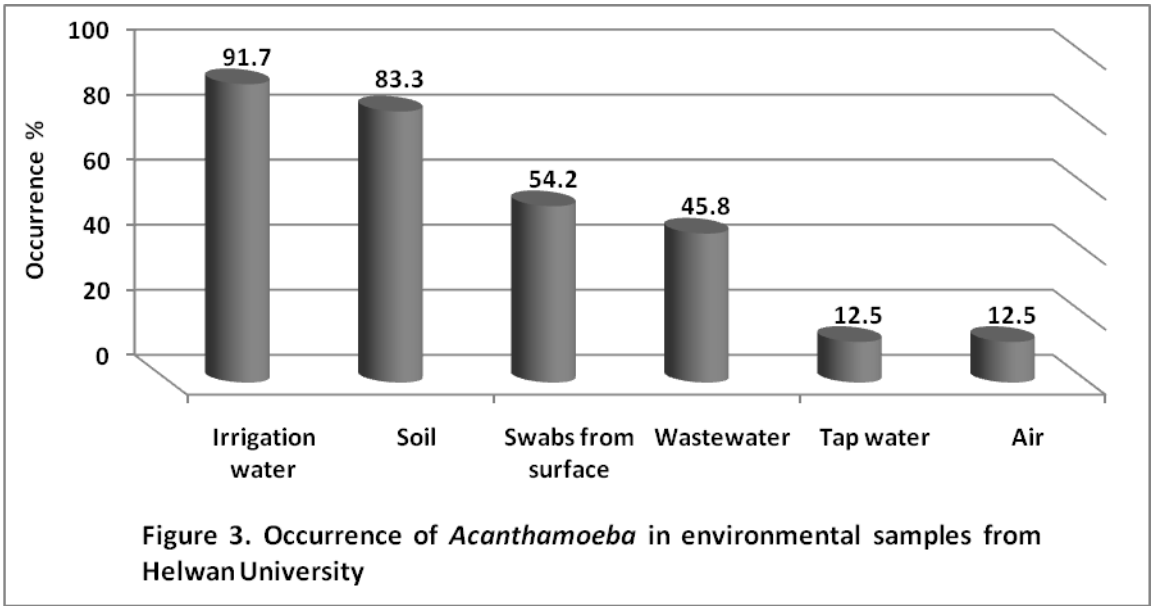

Figure 3. Occurrence of *Acanthamoeba* in environmental samples from Helwan University

Table 3. Comparison between the distribution of *Acanthamoeba* among different sampling sources

ANOVA summary	
F	**16.8**
P value	**<0.0001**
P value summary	********
Significant difference among means (P < 0.05)	**Yes**
R square	**0.3784**

Results of the present work declared that spring season recorded the highest appearance of *Acanthamoeba*. Irrigation water, soil and swabs from surface samples in spring season had the highest percentage of *Acanthamoeba* (100%). Also irrigation water samples recorded full appearance in summer season. The highest occurrence of *Acanthamoeba* in irrigation water samples was observed in spring and summer seasons (100%), and then it decreased to be 83% in winter and autumn. The highest occurrence of *Acanthamoeba* in

soil samples was observed in spring season (100%), and then it decreased to be 83% in winter, while it reached to the lowest occurrence 67% in autumn. The highest occurrence of *Acanthamoeba* in swabs from surfaces samples was observed in spring season (100%), and then it represented 50% in summer and autumn, while it reached the lowest occurrence (17%) in winter. The highest occurrence of *Acanthamoeba* in wastewater samples was observed in winter season (67%), and then it represented 50% in spring and autumn, while it reached the lowest occurrence (33%) in summer. On the other hand, the occurrence percentage of *Acanthamoeba* in tap water samples was the same in spring, autumn and winter(represented by 17% for each), while it was disappeared in summer. Concerning air samples, the highest occurrence of *Acanthamoeb*a was recorded in summer season, while they disappeared in spring, autumn and winter (Figure 4).

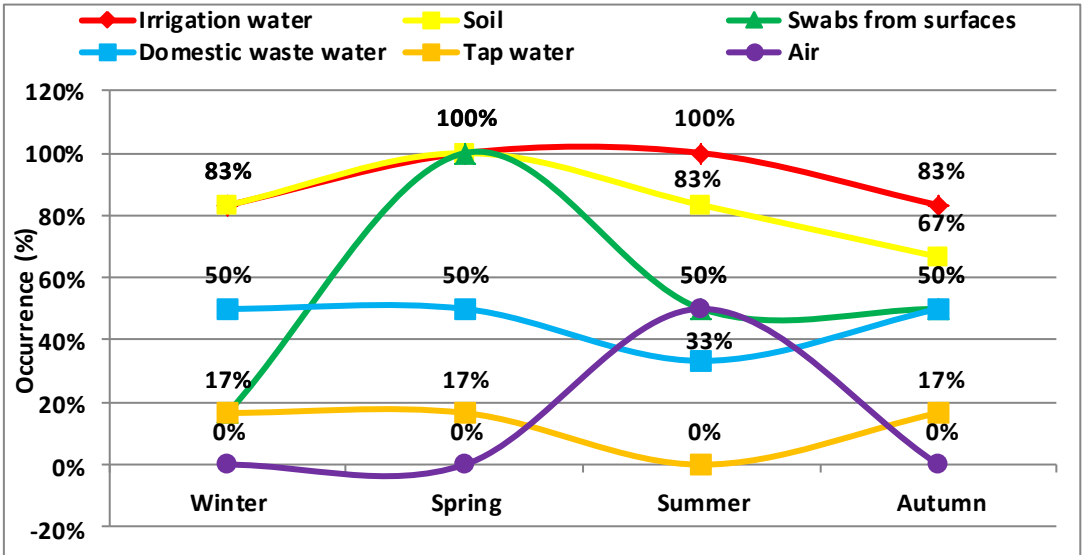

Figure 4: Seasonal variation of *Acanthamoeba* Spp. in environmental samples from Helwan university

Other workers in Egypt found that winter followed by autumn showed the peak for *Acanthamoeba* species in all inspected governorates. In Faiyum and Qalyubia governorates, winter was the highest season for occurrence of *Acanthamoeba* species (55.5 and 33.3%, respectively). Although *Acanthamoeba* species have been identified throughout the year, wet seasons showed the highest occurrence **(Gad *et al.*, 2019).**
Acanthamoeba species, the most common free-living amoebae, have been isolated from a wide range of environments particularly water. These amoebae have been reported to feed by phagocytosis on bacteria, fungi, and algae **(Król-Turmińska and Olender, 2017; Chen *et al.*, 2018).** According to the previous reports**,** *Acanthamoeba* might serve as an environmental reservoir for viruses living in the same environment, such as *Mimi* virus, *Coxsackie* virus and *Adenovirus* **(Scheid and Schwarzenberger, 2012; Yousuf *et***

***al.*, 2017)**. Other workers demonstrated that the environmental isolate *Acanthamoeba mauritaniensis* genotype T4D, which was previously characterized as a non-pathogenic amoeba by **De Jockheere (1980)**, is able to produce and secrete serine proteases that can be involved in epithelial damage and in the alteration of TJ proteins **(Coronado-Velázquez et al., 2020).**

The seasonal variation of *Acanthamoeba* was noted, with a peak during summer months or warmer months either in clinical or water samples **(Page and Mathers, 2013; Gad and Al-Herrawy, 2016).** Other workers found that *Acanthamoeba* genotype T4 was the most predominant genotype in tap water in Egypt. Regardless of the disinfectant applied at a drinking water utility, cross-contamination can occur throughout the water distribution system due to cavitations; therefore, the use of secondary disinfectants in distribution systems is required **(Gall *et al*., 2015).** Recently, among the free-living amoebae (FLAs) microbiome, the highly pathogenic *Helicobacter pylori* bacteria were detected alive from the inside of these amoebae, pointing out that FLAs are carriers of these pathogens which can reach humans and cause a public health concern **(Moreno-Mesonero *et al*., 2020).**

CONCLUSION

The relatively high prevalence of *Acanthamoeba* species in tap water presents a public health hazards which reflect the importance of the presence of a regular monitoring plan for the water sources in Egypt. Generally, this work has underlined the need for additional deeper studies to investigate the actual genotypes of free-living amoebae and how they could be eliminated.

ACKNOWLEDGMENT

The authors are very grateful to Dr. Mahmoud Afw Gad, Associate Professor in Environmental Parasitology, National Research Centre, Egypt for his kind assistance in statistical analysis of this study.

REFERENCES

Abd El Wahab, W.M.; El-Badry, A.A. and Hamdy, D.A. (2018). Molecular characterization and phylogenetic analysis of *Acanthamoeba* isolates in tap water of Beni-Suef, Egypt. Acta Parasitol. 63(4): 826–834.

Al-Herrawy, A.Z. (1992). In vitro cultivation of agents of amoebic meningoencephalitis isolated from water and sewage. Ph.D. thesis. Faculty of Veterinary Medicine, Alexandria University, Egypt.

Al-Herrawy, A.Z.; Marouf, M.A. and Gad, M.A. (2017). *Acanthamoeba* species in tap water, Egypt. Inter. J. Pharma. Clin. Res. 9(1): 21-25.

American Public Health Association (2017): Standard methods for the examination of water and wastewater. 23th ed. APHA, WEF and AWWA, Washington DC.

Burger, G.; Plante, I.; Lonergan, K.M. and Gray, M.W. (1995): The mitochondrial DNA of the amoeboid protozoon, *Acanthamoeba castellanii*: complete sequence, gene content and genome organization. J. Mol. Biol., 245: 522–537.

Castellani, A. (1930). An amoeba found in culture of yeast: preliminary note. J. Trop. Med. Hyg., 33: 160.

Chen, W,; Deng, S.; Gao, M.; Jiang, C. and Li, R. (2018). *Acanthamoeba* keratitis, Internat. Ophthalmol. Clinic. Springer Nature. doi:http://dx.doi.org/10. 1097/IIO.0b013e318036bcf4. ISBN 978-981-10-5212-5.

Chung, D.I.; Yu, H.S.; Hwang, M.Y.; Kim, T.H.; Kim, T.O.; Yun, H.C. and Kong, H.H. (1998). Subgenus classification of *Acanthamoeba* by riboprinting. Korean J. Parasitol. 36: 69-80.

Coronado-Velazquez, D; Silva-Olivares, A.; Castro-Munozledo, F.; Lares-Jimenez, L.F.; Rodriguez-Anaya, L.Z.; Shibayama, M. and Serrano-Luna, J. (2020). *Acanthamoeba mauritaniensis* genotype T4D: An environmental isolate displays pathogenic behavior. Parasitol. Internat. 74: 1-11.

Coşkun, K.A.; Özçelik, S.; Tutar, L.; Elaldı, N. and Tutar, Y. (2013). Isolation and identification of free-living amoebae from tap water in Sivas, Turkey. Biomed. Res. Int. 2013:1-8.

De Jonckheere, J.F. (1980). Growth characteristics, cytopathic effect in cell culture, and virulence in mice of 36 type strains belonging to 19 different *Acanthamoeba* spp, Appl. Environ. Microbiol. 39 (4): 681–685.

Derda, M.; Winiecka-Krusnell, J.; Linder, M.B. and Linder, E. (2009). Labeled *Trichoderma reesei* cellulase as a marker for *Acanthamoeba* cyst wall cellulose in infected tissues. Appl. Environ. Microbiol. 75(21): 6827–6830.

Dudley, R.; Jarroll, E.L. and Khan, N.A. (2009). Carbohydrate analysis of *Acanthamoeba castellanii*. Exp Parasitol. 122(4): 338–343.

Dykova, I.; Lom, J.; Schroeder-Diedrich, J. M.; Booton, G.S. and Byers, T.J. (1999). *Acanthamoeba* stains isolated from organs of freshwater fishes. J. Parasitol., 85: 1106-1113.

Gad, M.A. and Al-Herrawy, A.Z. (2016). Real time PCR detection of *Acanthamoeba* species in the Egyptian aquatic environment. Int. J. Pharma. Clin. Res. 8(11): 1510–1515.

Gad, M.A.; Allayeh, A.K.; Elmahdy, E.M.; Shaheen, M.N.; Rizk, N.M.; Al-Herrawy, A.Z.; Saleh, F.R. and Marouf, M.A. (2019). Genotyping and interaction reality of *Acanthamoeba*, enteric viruses and rotavirus in drinking water, Egypt. Egypt. J. Aqua. Biol. Fish., 23(2): 65-79.

Gall, A.M.; Marinas, B.J.; Lu, Y. and Shisler, J.L. (2015). Waterborne viruses: a barrier to safe drinking water. PLoS Pathogens 11: e1004867.

Health Protection Agency (2004). *Isolation and identification of Acanthamoeba* species. National Standard Method W 17 Issue 2. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

Jeong, H.J. and Yu, H.S. (2005). The role of domestic tap water in *Acanthamoeba* contamination in contact lens storage cases in Korea. Korean J. Parasitol. 43(2): 47–50.

Khan, N.A. (2003): Pathogenesis of *Acanthamoeba* infections. Microb. Pathogen.,34: 277–285.

Kong, H.H. and Chung, D.I. (2002). A riboprinting scheme for identification of unknown *Acanthamoeba* isolates at species level. Korean J. Parasitol. 40: 25-31.

Król-Turmińska, K. and Olender, A. (2017). Human infections caused by free-living amoebae. Ann. Agric. Environ. Med. 24(2): 254–260.

Leiva, B.; Clasdotter, E.; Linder, E. and Winiecka-Krusnell, J. (2008). Free-living *Acanthamoeba* and *Naegleria* spp. amebae in water sources of León, Nicaragua. Rev. Biol. Trop. 56(2): 439–446.

Magistrado-Coxen, P.; Aqeel, Y.; Lopez, A.; John, R. Haserick, J.R.; Urbanowicz, B.R.; Catherine, E. Costello, C.E. and Samuelson, J. (2019). The most abundant cyst wall proteins of *Acanthamoeba castellanii* are lectins that bind cellulose and localize to distinct structures in developing and mature cyst walls. PLOS Neglect. Trop. Dis. 2019: 1-33.

Marciano-Cabral, F. and Cabral, G. (2003). *Acanthamoeba* spp. as agents of disease in humans. Clin. Microbiol. Rev., 16: 273-307.

Martinez, A.J. and Visvesvara, G.S. (1997). Free-living, amphizoic and opportunistic amoebae. Brain Pathol. 7: 583-598.

Moreno-Mesonero, L.; Hortelano, I.; Moreno, Y. and Ferus, M.A. (2020). Evidence of viable *Helicobacter pylori* and other bacteria of public health interest associated with free-living amoebae in lettuce samples by next generation sequencing and other molecular techniques. Internat. J. Food Microbiol. 318: 1-8.

Morsy, G.H.; Al-Herrawy, A.Z.; Elsenousy, W.M. and Marouf, M.A. (2016). Prevalence of free-living amoebae in tap water and biofilm, Egypt. Res. J. Pharm. Biol. Chem. Sci. 7(1): 752–759.

Onichandran, S.; Kumar, T.; Salibay, C.C.; Dungca, J.Z.; Tabo, H.A.L.; Tabo, N.; Tan, T.C.; Lim, Y.A.L.; Sawangjaroen, N.; Phiriyasamith, S.; Andiappan, H.; Ithoi, I.; Lau, Y.L. and Nissapatorn, V. (2014). Waterborne parasites: A current status from the Philippines. Parasit. Vector. 7(244): 1–8.

Page, F.C. (1988): A new key to freshwater and soil gymnamoebae. Freshwater Biological Association, Ambleside, UK.

Page, M.A. and Mathers, W.D. (2013). *Acanthamoeba* keratitis: a 12-year experience covering a wide spectrum of presentations, diagnoses, and outcomes. J. Ophthalmol. 2013: 1-6.

Preston, T.M.; Richards, H. and Wotton, R.S. (2001). Locomotion and feeding of *Acanthamoeba* at the water air interface of ponds. FEMS Microbiol. Lett., 194: 143-147.

Pussard, M. and Pons, R. (1977). Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida). Protistol., 13: 557- 598.

Sakran, T.F.; El-shahawy, G.A.; Shalaby, M.A.; Sabry, H.Y.; Matooq, P.M. and Elmallah, A.M. (2017). Detection rates of waterborne protozoa in water sources from Fayoum Governorate. Parasitol. United J. 10: 30–33.

Scheid, P. and Schwarzenberger, R. (2012). *Acanthamoeba* spp. as vehicle and reservoir of adenoviruses. Parasitol. Res. 111(1): 479–485.

Schroeder, J.M.; Booton, G.C.; Hay, J.; Niszl, I.A.; Seal, D.V.; Markus, M.B.; Fuerst, P.A. and Byers, T.J. (2001). Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoeba* from humans with keratitis and from sewage sludge. J. Clin. Microbiol. 39: 1903-1911.

Schuster, F.L. and Visvesvara, G.S. (2004). Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int. J. Parasitol., 34: 1001-1027.

Sriram, R.; Shoff, M. Booton, G.; Fuerst, P. and Visvesvara, G.S. (2008). Survival of *Acanthamoeba* cysts after desiccation for more than 20 years. J. Clinic. Microbiol. 46: 4045–4048**.**

Tawfeek, G.M.; Bishara, S.A.H.; Sarhan, R.M.; El Shabrawi Taher, E. and El Saady Khayyal, A. (2016). Genotypic, physiological, and biochemical characterization of potentially pathogenic *Acanthamoeba* isolated from the environment in Cairo, Egypt. Parasitol. Res. 115(5): 1871–1881.

Visvesvara, G.S. (2010). Amebic meningoencephalitides and keratitis: challenges in diagnosis and treatment. Curr. Opin. Infect. Dis. 23: 590-594.

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: 1–26.

Yoder, J.S.; Eddy, B.A.; Visvesvara, G.S.; Capewell, L. and Beach, M.J. (2010). The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. Epidemiol. Infect. 138: 968-975.

Yousuf, F.A.; Siddiqui, R. and Khan, N.A. (2017). Presence of rotavirus and free-living amoebae in the water supplies of Karachi, Pakistan. Rev. Inst. Med. Trop. Sao Paulo, 59:1-7.

ARABIC SUMMARY

توزيع عزلات ال *Acanthamoeba* المسببة للأمراض في بيئة جامعة حلوان ، مصر

هبه قطيط١ ، شحاته السباعي علوه١ ، أحمد زكريا الهراوي٢*

١- قسم علم الحيوان والحشرات – كلية العلوم – جامعة حلوان – مصر.

٢- قسم بحوث تلوث المياه – المركز القومي للبحوث – جيزه – مصر.

تمثل أفراد جنس ال *Acanthamoeba* أكثر أجناس الأميبات حرة المعيشة تواجدافي البيئة في جميع أنحاء العالم. ومعظم أفراد هذا الجنس يمكن أن تسبب التهاب الدماغ الأميبي الحبيبي والتهاب القرنية الأميبي لدى البشر وذلك لقدرتها علي إنتاج إنزيمات proteases التي تعتبر من أهم عوامل شراستها وضراوتها، إلي جانب قدرتها علي إيواء البكتيريا المسببة للأمراض والفطريات والفيروسات.

الهدف من هذه الدراسة هو تقييم وجود ال *Acanthamoeba* في بيئة جامعة حلوان ، مصر.

تم جمع ستة أنواع من العينات (ماء الصنبور ، مياه الري ، مياه الصرف الصحي ، مسحات من الأسطح ، التربة والهواء) ، وتمت معالجة هذه العينات وتركيزها واستزراعها علي بيئة الآجار غير المغذي. تم إعادة زرع العينات الإيجابية لل *Acanthamoeba* ، وتم تحديدها وتنقيتها والتعرف عليها مورفولوجيا وتأكيدها بواسطة PCR باستخدام البادئ الخاص بجنس ال *Acanthamoeba*.

أظهرت النتائج التي تم الحصول عليها أنه تم الكشف عن جنس ال *Acanthamoeba* في ٩١.٧ ، ٨٣.٣ ، ٥٤.٢ ، ٤٥.٨ ، ١٢.٥ و ١٢.٥ ٪ من مياه الري ، التربة ، المسحات ، مياه الصرف الصحي ، عينات مياه الصنبور والهواء ، على التوالي. أثبتت أنواع ال *Acanthamoeba* المعرفة مورفولوجيا أنها تتبع جنس *Acanthamoeba* عند اختبارها باستخدام تقنية ال PCR. إحصائيا ، كان لمصدر أخذ العينات ارتباط قوي وكبير على انتشار ال *Acanthamoeba*. تم تسجيل أعلى ظهور لل *Acanthamoeba* في موسم الربيع لعينات مياه الري والتربة ومسحات السطوح.

الخلاصة أن ارتفاع انتشار الأميبات التابعة لجنس ال *Acanthamoeba* في مياه الري والتربة يشكل مخاطر صحية عامة للطلاب والعاملين في جامعة حلوان.