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Research Article

Acanthamoeba Species in Tap Water, Egypt

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ABSTRACT

Genus *Acanthamoeba* causes 3 clinical syndromes amebic keratitis, granulomatous amebic encephalitis and disseminated granulomatous amebic disease (eg, sinus, skin and pulmonary infections). A total of 144 tap water samples were collected from Giza governorate, Egypt. Samples were processed for detection of *Acanthamoeba* species using non-nutrient agar (NNA) and were incubated at 30ºC. The isolates of *Acanthamoeba* were identified to species level based on the morphologic criteria. Molecular characterization of the *Acanthamoeba* isolates to genus level was performed by using PCR. The obtained results showed that the highest occurrence percentage of *Acanthamoeba* species in water samples was observed in summer season (38.9%), then it decreased to be 30.6% in spring and 25% in each of autumn and winter. PCR analysis showed that 100% of 43 *Acanthamoeba* morphologically positive samples were positive by genus specific primer. In the present study eight species of *Acanthamoeba* can be morphologically recognized namely *Acanthamoeba triangularis, Acanthamoeba echinulata, Acanthamoeba astronyxis, Acanthamoeba comandoni, Acanthamoeba griffini, Acanthamoeba culbertsoni, Acanthamoeba quina* and *Acanthamoeba lenticulata.* In conclusion, the most common *Acanthamoeba* species in tap water was *Acanthamoeba comandoni.*

Keywords: *Acanthamoeba*, Morphology, PCR, Tap water, Egypt.

INTRODUCTION

There is a worldwide worry that all the total populace ought to have entry to safe drinking water. Indeed, even in the 21st century, there are many individuals without access to safe water, in amount and additionally quality for the basic needs[1]. One of the vital issues in water pollution is the occurrence of pathogenic amoebae in tap or drinking water[2]. The presence of these pathogens needs to be monitored in order to assess the human risk from a several of illnesses ranging from keratitis, gastroenteritis, and infections of the lungs and skin to more serious illnesses such as primary amoebic meningoencephalitis (PAM), aseptic meningitis and granulomatous amoebic encephalitis (GAE)[3]. Free-living amoebae are ubiquitous in aquatic environments and are aerobic, eukaryotic, mitochondriate protists[3]. They are frequently alluded to as amphizoic amoebae because of their capacity to live unreservedly without a host notwithstanding having the ability to attack a host and live as parasites[3,4]. People are consistently presented to these amoebae because of their pervasive event in the earth. These microorganisms can likewise be found in drinking water[5]. Genus *Acanthamoeba* is a member of free-living amoebae, individuals from which can bring about a conceivably blinding keratitis in people[6]. *Acanthamoeba* life cycle contains dormant cyst and feeding, replicating trophozoite stages[7]. The *Acanthamoeba* cysts resistance to most of antimicrobial agents makes *Acanthamoeba* keratitis one of the most difficult ocular infections to treat with a mean treatment time of over 5 months[8,9]. *Acanthamoeba* cysts can withstand extremes of desiccation, temperature, and disinfection,[10] these organisms were isolated from soil, rivers, mud, lakes, ponds, water cooling towers, tap water, chlorinated bathing pools and the atmosphere[10,11]. *Acanthamoeba polyphaga, Acanthamoeba castellanii, Acanthamoeba palestinensis, Acanthamoeba culbertsoni, Acanthamoeba astronyxis, Acanthamoeba rhysodes, Acanthamoeba divionensis, Acanthamoeba hatchetti, Acanthamoeba healyi* and *Acanthamoeba griffini* have all been implicated in human infections[12]. So, the objective of the present study was to assess the prevalence of *Acanthamoeba* species in Giza governorate tap water in Egypt.

MATERIALS AND METHODS

A total of 144 tap water samples were collected from Giza governorate during the period of one year. Twelve water samples (1liter volume each) were collected every month. Samples were separately collected in a clean, dry and autoclavable polypropylene containers then sent to the laboratory where they were processed at the same day of collection. Samples were transported at ambient temperature[13,14]. One liter of each sample was filtered through a nitrocellulose membrane filter (0.45 µm pore size and 47 mm diameter) using a stainless steel filter holder connected with a suction pump. Filtration was stopped just before drying of the membrane[13,14]. After filtration process, the membrane was inverted face to face on the surface of a non-nutrient agar (NNA) plate seeded with heat-killed *Escherichia coli*. The plate was wrapped with parafilm and incubated at 30ºC for the cultivation of free-living amoebae[14,15]. Incubated plates were daily

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Table 1: Prevalence of *Acanthamoeba* spp in tap water.

Seasons	Total number of examined samples	Total positive culture for FLA		Positive samples for *Acanthamoeba* by culturing method		Positive samples for *Acanthamoeba* by PCR
		No	%	No	%	No
Winter	36	12	33.3	9	25	9
Spring	36	15	41.7	11	30.6	11
Summer	36	19	52.8	14	38.9	14
Autumn	36	18	50	9	25	9
Total	144	64	44.4	43	29.9	43

Table 2: Distribution of the isolated species of *Acanthamoeba* in tap water.

Acanthamoeba species	+ve in tap water samples
Acanthamoeba astronyxis	9
Acanthamoeba comandoni	10
Acanthamoeba echinulate	6
Acanthamoeba triangularis	9
Acanthamoeba quina	3
Acanthamoeba culbertsoni	1
Acanthamoeba griffini	4
Acanthamoeba lenticulata	1

examined by inverted microscope (Olympus CXK 41, Japan) for 7 days for the presence of any amoebic growth. All cloned amoebae were evaluated with morphological criteria according to page key[7]. The morphologically positive isolates of *Acanthamoeba* were subjected to molecular characterization. *Acanthamoeba* cysts walls were perforated by three consecutive applications of freezing and thawing in liquid nitrogen, followed by incubation in a water bath at 100°C for 10 minutes. *Acanthamoeba* DNA was then extracted using Ez-10 spin column fungal genomic DNA mini-preps kit (Bio Basic Canda inc.). The extracted DNA were identified by PCR using genus specific primers; AcantF900 (5′-CCC AGATCG TTT ACC GTG AA-3′) and AcantR1100 (5′-TAA ATA TTA ATG CCC CCAACT ATC C-3′) to amplify 18S rRNA gene fragment of approximately 180 bp (differing by a few bases depending on the species)[16]. Amplification of DNA was performed using Maxima Hot Start Green PCR master mix (Thermo Scientific). The amplification of the respective fragment was visualized by ethidium bromide staining of an agarose electrophoresis gel and compared to DNA 100 plus bp (Gene Direx, China).

RESULTS

By examination of 144 water samples collected from different drinking water pipe lines revealed that the percentage of free-living amoebae was 44.4%. Concerning seasonal variation, the highest occurrence (52.8%) of free living amoebae was recorded in summer season. The rate of detection of free-living amoebae decreased gradually to be 50, 41.7 and lastly 33.3% in autumn, spring and winter, respectively. The highest occurrence percentage of *Acanthamoeba* species in water samples was observed in summer season (38.9%), then it decreased to be 30.6% in spring and 25% in each of autumn and winter. PCR analysis showed that 100% of 43 *Acanthamoeba* morphologically positive samples were positive by genus specific primer (Table 1 & figure 2).

Morphological characteristics of isolated Acanthamoeba species

Different species of the genus *Acanthamoeba* were identified according to the shape and size of cysts in addition to the number, shape, size and arrangement of the cyst pores. In the present study eight species of *Acanthamoeba* can be morphologically recognized namely *A. triangularis, A. echinulata, A. astronyxis, A. comandoni, A. griffini, A. culbertsoni, A. quina, A. lenticulata.*

Acanthamoeba astronyxis

Acanthamoeba astronyxis was isolated from 9 tap water samples. Its cyst diameter reached 19-22 µm. Ectocyst was smoothing circular or nearly so. Endocyst was usually stellate with mainly 5 rays ending with pores. The number of cyst pores reached 4-6. Tips of rays were approximately at general level of ectocyst, but not in depressions. All rays of endocyst usually contacted ectocyst in approximately the same plane. These isolates belonged to group (I) and didn't grow at 37°C (Table 2 & figure 1).

Acanthamoeba comandoni

Acanthamoeba comandoni was isolated from 10 tap water samples. Cyst diameter of *Acanthamoeba comandoni* reached 20-25 µm**.** Endocyst had a stellate form with mainly 6-9 rays**.** Ectocyst was delicate and not wrinkled encircling endocyst. Rays of endocyst contact ectocyst in several planes. The number of cyst pores reached 6-9 (Table 2 & figure 1).

Acanthamoeba echinulate

Acanthamoeba echinulata was isolated from 6 tap water samples. Cysts of *Acanthamoeba echinulata* have the same characters as *A. comandoni* except that the number of pores was up to 10 or more. Cyst diameter of *Acanthamoeba echinulata* was up to 25 µm or more (Table 2 & figure 1).

Acanthamoeba triangularis

Acanthamoeba triangularis was isolated from 9 tap water samples. Endocyst which could be stellate, polygonal and triangular. Ectocyst was thick wrinkled and corrugated but is not spherical. Rays of endocyst are broad and slightly curved. The average number of pores was 3 or 4. The diameter of cysts was 13 µm. This isolate belonged to group (II) and was able to survive at 40°C. (Table 2 & figure 1).

Acanthamoeba quina

Acanthamoeba quina was isolated from 3 tap water samples. Endocyst are globular, ovoid, sometimes quadrangular, pentagonal or even pear-shaped. The

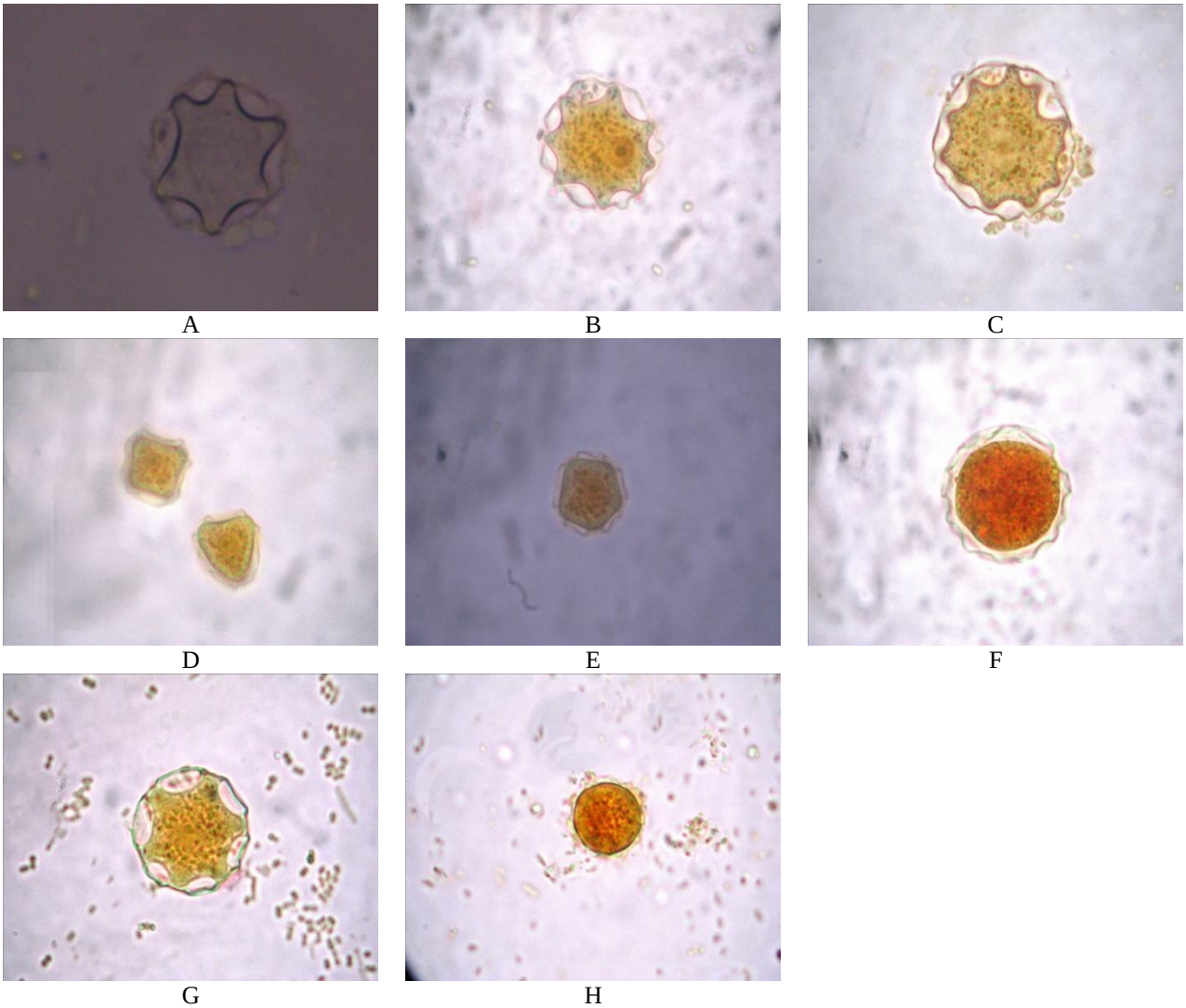

Figure 1: Photomicrograph showed different *Acanthamoeba* cysts stained with Lugol's iodine. iodine; A, *Acanthamoeba astronyxis* cyst; B, *Acanthamoeba comandoni* cyst; C, *Acanthamoeba echinulate* cyst; D, *Acanthamoeba triangularis* cyst; E, *Acanthamoeba quina* cyst; F, *Acanthamoeba culbertsoni* cyst; G, *Acanthamoeba griffini* cyst; H, *Acanthamoeba lenticulata* cyst.

ectocyst is slightly pleated, thinner than the endocyst and applied on endocyst. This strain belonged to group (II). The diameter of cysts was 12 µm. The average number of pores was 4 or 5 (Table 2 & figure 1).

Acanthamoeba culbertsoni

Acanthamoeba culbertsoni was isolated from 1 tap water samples. This isolates belong to group (III) and grows poorly at 23°C, well at 37°C and Cyst diameter reached 15-16-18µm**.** Ectocyst was more noticeably wrinkled and is thinner than the endocyst**.** Endocyst was nearly rounded or with only slight angles. In general it is difficult to spot the pores on living cysts but number of pores was 5-6 per cyst (Table 1 & figure 1).

Acanthamoeba griffini

Acanthamoeba griffini was isolated from 4 tap water samples. These isolates belong to group (Π) and Cyst diameter reached 14µm.Endocyst usually with pronounced polygonal or stellate shape with mainly 6 rays which appearing possibly wider at the tip. The ectocyst was transparent and does not apply on the endocyst. The six rays are arranged into two isosceles triangles (Table 2 & figure 1).

Acanthamoeba lenticulata

Ectocyst was not predominantly stellate. A stellate ectocyst was closely following endocyst contour. Endocyst nearly rounded or with only slight angles. Cyst diameter was 11-13µm.The endocyst had 6 pores (Figure 20). This isolate belonged to group (III). It was isolated from 1 tap water samples (Table 2 & figure 1).

DISCUSSION

The present study is dealt with the natural distribution and predominance of *Acanthamoeba* in tap water in Giza governorate, Egypt. To the best of our knowledge, little previous studies concerning *Acanthamoeba* in tap water in Egypt were published[17,18]. The results of the present study showed that the prevalence of *Acanthamoeba* was 29.9% in 144 examined tap water samples. And the most common species in the present study was *Acanthamoeba comandoni* followed by *Acanthamoeba astronyxis* and *Acanthamoeba*

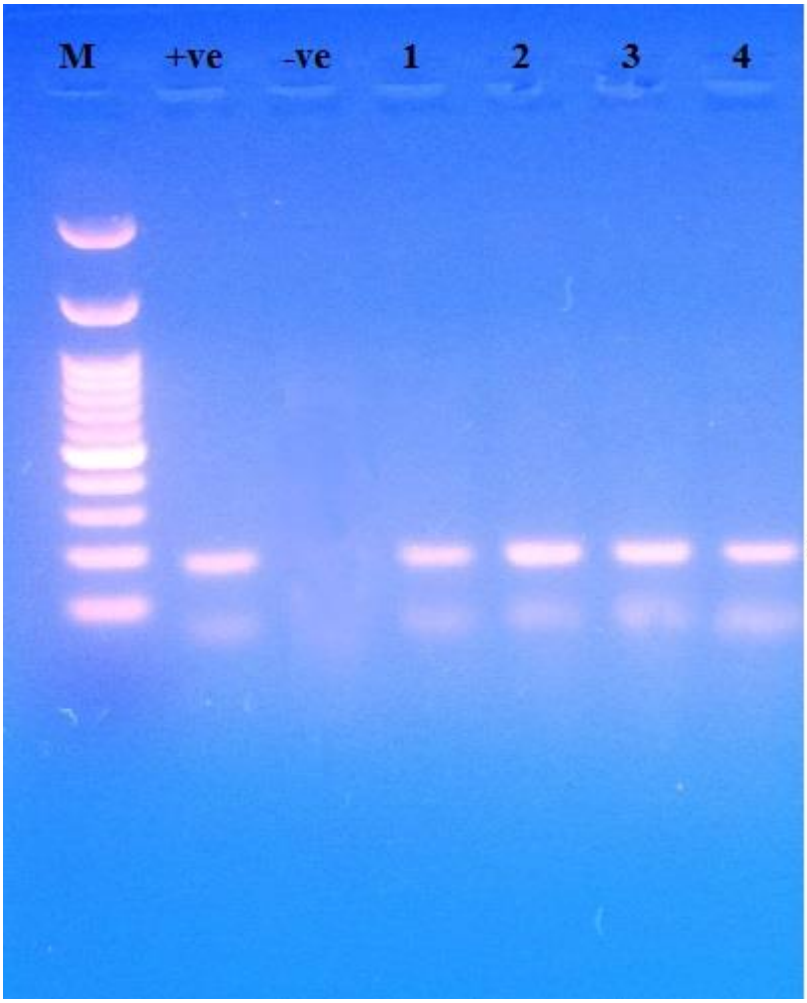

Figure 2: Agarose gel electrophorisis for PCR amplified product of DNA from *Acanthamoeba* spp. Lane 1: 100 plus DNA ladder; Lane 2: Control Positive; Lane 3: Control negative; lanes 4, 5, 6 and 7: Positive samples.

triangularis. In other study in Egypt, The prevalence of *Acanthamoeba* spp. was detected in a lower percentage 8.3 % in tap water by real time PCR[19]. In another work, *Acanthamoeba* species were identified by microscopy in 237 (51%) of households samples from Ohio, USA. The most common species identified was *A. hatchetti*, found in 165 (35%) of households, followed by *A. polyphaga* in 155 (33%) of households[20]. In Pakistan, about 30% of the examined tap water samples were positive for *Acanthamoeba* spp.[2]. In India, Lorenz-Morales *et al*.[21] detected *Acanthamoeba* spp. in 36.1% of tap water samples. Other researchers in Nicaragua found a lower percentage (21%) *Acanthamoeba* in drinking water samples[22]. The differences in percentages of detection of *Acanthamoeba* in different study may be due to difference in geographic areas. Interestingly there is no single case of *Acanthamoeba* keratitis or *Acanthamoeba* granulomatous encephalitis was reported in Egypt, despite the high prevalence of *Acanthamoeba* in the drinking water. This is probably due to lack of awareness and the difficulty in diagnosis. The results of the current study showed that the highest occurrence percentage of *Acanthamoeba* species in water samples was observed in summer season (38.9%), then it decreased to be 30.6% in spring and 25% in each of autumn and winter. Other researchers in USA found that the increase in detections from spring to summer months was seen in all genera except for *Naegleria*. There was no appreciable difference in detections across the years of the study[20]. An increase in *Acanthamoeba* during the summer had been found in studies concerning the distribution of FLAs in Oklahoma, Virginia and South Carolina waters[23,24]. These results were nearly similar to our results. Results of the current study were also supported by a previous report that *Acanthamoeba* species are most prevalent in late summer in the aquatic environment[25]. The density and diversity of free-living amoebae at point of use were influenced by environmental temperature variations[5,26] and also increased during the summer months[27]. Similarly, the diversity of FLAs differed between the seasons. Specifically, in one study, *Acanthamoeba* species were isolated throughout the year, including temperatures as low as 0.5°C[5].

CONFLICT OF INTEREST

Conflict of interest declared none.

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