Evaluation of Acanthamoeba Species from Tehran Surface Water Sources Using Real Time PCR Method

Mafi M*, Niyyati M, Haghighi A

Abstract*— *Acanthamoeba spp. is the ubiquitous potentially pathogenic free-living amoebae in nature such as water sources. Recently *Acanthamoeba keratitis* (Ak) continue to rise in Iran. Most of patients report a history of contact with water sources before the onset of disease. The main aim of the present study was to determine the occurrence of *Acanthamoeba* spp. in the recreational water sources of Tehran, Iran using morphological and molecular based tests. A total of 70 surface water samples were collected from environmental sources, including parks pools and swimming pools. The samples were filtrated and transferred to non-nutrient agar plates seeded with Escherichia coli and incubated for 2 to 7 days at 30°C or 42°C. The plates were examined by microscopy to morphologically identify *Acanthamoeba spp*. Following DNA extraction, PCR was used to confirm the microscopically identification. A total of 11 out of 35 samples of parks pools and 3 out of 35 of swimming pools were positive for Acanthamoeba species based on the morphological criteria, and all were confirmed by PCR method. The significant frequency of *Acanthamoeba spp* in surface water sources of Tehran is of importance to beconsidered from public health point of view by authorities.

***Keywords*— Acanthamoeba spp,** *Surface Water Sources, PCR, Tehran, Iran.*

I. Introduction

FREE-LIVING amoebae (FLA), ubiquitous and widely distributed protozoa, feed on bacteria, algae, fungi, and small organic particles and are adaptable to their environment [1]. Among them, *Acanthamoeba spp*. are an opportunistic amphizoic protozoa, commonly found in the environment. Researchers showed that *Acanthamoeba* can be found in different environmental sources such as water, soil, sewage, and swimming pool[2],[3]. *Acanthamoeba* species are classified into three morphologic groups. Group I has large cysts with rounded outer walls (ectocysts) that are clearly separated from the inner walls (endocysts). Group II cysts are smaller, with variable endocyst shapes. Group III cysts are smaller than Group II cysts, with poorly separated walls. The major human pathogens belong to Group II[4] –[7]. Also some strains can cause granulomatous amoebic encephalitis (GAE). Thus, several species of *Acanthamoeba* has different clinical sign with the potential to cause a corneal infection termed*Acanthamoeba* keratitis (AK) [8]-[11]. Amoebic keratitis (AK) infection can occur through use of the contaminated contact lenses with non-sterile water or through bathing or swimming in a contaminant water [8],[9]. The presence of *Acanthamoeba* in recreational water sources may represent a health risk to both immunocompromised and immunocompetent individuals [12] and they are resistant to extreme conditions of temperature, pH, and exposure to various chemicals [4], [7]. An increase in the number of intracerebral infections caused by worldwide has been reported [13]. The taxonomy and classification of these protozoa are still under revision by the successful application of molecular techniques [14], [15]. Evolutionary studies have led to the identification of at least 17 genotypes (T1–T17) based on rRNA gene sequencing. AmongAcanthamoeba genotypes, genotype T4 is the most prevalent type causing disease in human. [16],[1 7]. Indeed, Acanthamoeba spp. are an opportunistic causative agent of nasopharyngeal and skin infections. Also some strains can cause granulomatous amoebic encephalitis (GAE). Thus, several species ofAcanthamoeba has different clinical sign with the potential to cause a corneal infection termedAcanthamoeba keratitis (AK) [8]-[11]. Amoebic keratitis (AK) infection can occur through use of the contaminated contact lenses with non-sterile water or through bathing or swimming in a contaminant water[8], [9]. Recently, AK is rising in Iran and the world [18]. The presence of Acanthamoeba in water, soil, dust, cow faeces, and swimming pool has been shown in Iran [19]. Acanthamoeba T4,T3 genotype was isolated from biofilms and dust sources from hospitals [19]. Additionally, Acanthamoeba have been isolated from tap waters of the hospitals in Iran [20]. Since there was no information regarding the distribution of *Acanthamoeba* in recreational water sources, The main aim of the present study was to determine the occurrence of *Acanthamoeba* spp. in the recreational water sources of Tehran, Iran using morphological and molecular based tests.

II. Material and Methods

Totally, 70 samples (35 of parks pools and 35 of swimming pools) were collected from different localities of Tehran. The samples were examined in the laboratory of Protozoology Unit, Department of Parasitology, Shahid Beheshti University of Medical Sciences, Iran. The samples were filtrated and transferred to non-nutrient agar plates seeded with Escherichia coli and incubated for 2 to 7 days at 30°C or 42°C. The plates were examined by microscopy to morphologically identify *Acanthamoeba spp*. Following DNA extraction, PCR was used to confirm the microscopically identification.

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III. Results

A total of 11 out of 35 samples of parks pools and 3 out of 35 of swimming pools were positive for Acanthamoeba species based on the morphological criteria, and all were confirmed by PCR method (Figure I and Table I and Table II).

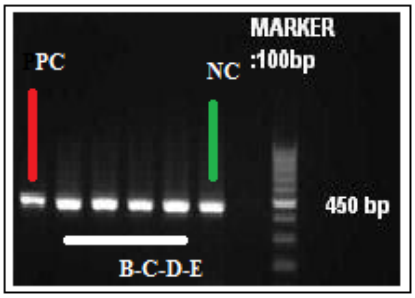

Fig. 1 PCR- Product of recreational water sources sample. M: Molecular weight marker(100 bp), PC: Positive Control, NC: Negative Control B-C-D-E: recreational water sources

TABLE I
DISTRIBUTION OF POSITIVE SAMPLES FROM DIFFERENT AREAS OF THE PARK PONDS IN TEHRAN, IRAN

sampling place	Isolated called positive	PCR	Morphology
Esteehlal park	*MN(AC)7-SHB	+	External wall with double amoebic cysts, irregular
Shaghaveeh park	MN(AC)10-SHB	+	External wall with double amoebic cysts, irregular
Nahgolbalag he park	MN(AC)14-SHB	+	External wall with double amoebic cysts, irregular
Yasaman park	MN(AC)18-SHB	+	External wall with double amoebic cysts, irregular
Nabovat park	MN(AC)21-SHB	+	External wall with double amoebic cysts, irregular
shafaeh park	MN(AC)25-SHB	+	External wall with double amoebic cysts, irregular
Farahzad park	MN(AC)30-SHB	+	External wall with double amoebic cysts, irregular
Ghazal park	MNAC)33-SHB	+	External wall with double amoebic cysts, irregular

TABLE II
DISTRIBUTION OF POSITIVE SAMPLES IN POOLS OF DIFFERENT AREAS OF TEHRAN, IRAN

sampling place	Isolated called positive	PCR	Morphology
Takhti pool	*MN(AC)7-SHB	+	External wall with double amoebic cysts, irregular
Firozeh pool	MN(AC)10-SHB	+	External wall with double amoebic cysts, irregular
Ghivanori pool	MN(AC)14-SHB	+	External wall with double amoebic cysts, irregular

IV. Discussion

This study indicated the present of *Acanthamoeba* spp. in recreational water sources in Tehran, Iran. Many studies have reported the presence of *Acanthamoeba* in drinking water, swimming pools, and rivers. These water sources have an obvious role in prevalence *Acanthamoeba* keratitis among people. Since *Acanthamoeba* has an extensive distribution, it is expected that individuals have exposure to the protozoa [22], [23]. Previous studies have shown that many *Acanthamoeba* isolated from tap-water sources might have some pathogenic ability [24]. the result of current research shows that *Acanthamoeba* is capable of swimming in water with disinfectant survive and cause morbidity in patients with immune deficiency and the young and children. Out of 70 water samples, 14 (20%) were positive for *Acanthamoeba* trophozoites and cysts according to morphological criteria. This finding showed the risk of being affected by *Acanthamoeba* in recreational water sources. *Acanthamoeba* spp. isolated from the water in Tehran mostly had genotypes belonged to T4[25]. High percentage of *Acanthamoeba* spp. in water, is a hygienic risk for public health especially for individuals with immune deficiency situation and use in recreational water sources[26].

PCR analysis and sequencing of isolates in this study revealed the existence of T4 genotypes in water sources. This finding is in accordance with other researches in Iran. Indeed, the presence of *Acanthamoeba* spp. in water where human activity is high may cause the infection in contact lens wearers [27].

V. Conclusion

Presence of *Acanthamoeba* in recreational water sources is of concern for high risk people. The significant frequency of *Acanthamoeba spp* in surface water sources of Tehran is of importance to be considered from public health point of view by authorities.

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