



## The *Acanthamoeba* spp. in Water Sources from Zanjan Province, Northwest of Iran

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### ABSTRACT

**Background:** The genus of *Acanthamoeba* is an opportunistic protozoan parasite with a worldwide distribution where it is able to cause fatal granulomatous amoebic encephalitis (GAE) and amoebic keratitis (AK). This organism inhabits in the wide range of natural and man-made aquatic environments. The present study was carried out to evaluate the presence of *Acanthamoeba* spp. in the various water resources of Zanjan province, northwest Iran, using both morphological and molecular methods.

**Methods:** The Water samples were randomly collected from 30 water sources in different parts of Zanjan, Iran, between April 2015 and May 2016. Then, the samples were cultured on non-nutrient agar and the *Acanthamoeba* genus identified by morphological characters. The polymerase chain reaction (PCR) was performed using the 18S rRNA gene as a molecular marker.

**Results:** The obtained data showed that, out of the 60 water samples collected, 30 (50%) were positive for *Acanthamoeba* spp. According to morphological and molecular approaches.

**Conclusion:** The present investigation is the first report of the distribution of *Acanthamoeba* spp. in the various water sources of Zanjan province, gives baseline knowledge regarding water contamination with *Acanthamoeba* spp. in these areas and emphasizes the necessity of more attention to water sources in order to prevent infections associated with *Acanthamoeba* spp.

## 1. Introduction

The genus of *Acanthamoeba* is a free living amoeba which is an important opportunistic parasite with a cosmopolitan distribution [1, 2].

This amphizoic organism has been isolated from air, soil, dust, sewage, sediments, contact lenses as well as the clinical specimens and is particularly plentiful in water [3, 4]. The genus of

*Acanthamoeba* has two developmental stages in its life cycle trophozoite and cyst, [5]. The trophozoite, is an active form, which the genus of *Acanthamoeba* is able to feed, grow, move and reproduce, and the cyst, is a non-active form. The double-layered coat cyst of this amoeba is resistant to disinfectants, antibiotics, UV radiation, and the chemical agents. Furthermore, it can survive as

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well at  $-2^{\circ}\text{C}$  to  $45^{\circ}\text{C}$  [6-9]. The pathogenic genotypes of *Acanthamoeba* are the etiological agents of granulomatous amoebic encephalitis (GAE), skin, lung, kidney, liver, spleen, prostate and uterus infections in the immunocompromised individuals.

Also some strains can cause amoebic keratitis in the healthy people who are soft contact lens users or have a history of corneal injury [10-13].

Moreover, the *Acanthamoeba* can carry and transmit a large number of microorganisms including the *Listeria monocytogenes*, the *Escherichia coli* serotype O157, *Burkholderia cepacia*, *Vibrio Cholerae*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Chlamydia pneumoniae*, *Coxiella burnetii*, *Legionella* spp, *Mycobacterium* spp, *Coxsackie virus*, *Echovirus* and *Adenovirus* [9, 14-16]. Thus, the *Acanthamoeba* plays both direct and indirect harmful roles in human's health [17, 18].

During the past years, the *Acanthamoeba* keratitis cases are regularly reported in the medical centers exist in Iran [19]. Moreover, some researchers have showed the presence of the undistinguished encephalitis in immunocompromised patients in the country [20].

The studies have demonstrated that the aquatic environments are among the most important sources for the transmission of *Acanthamoeba* spp to human's body [7, 13, 21]. The occurrence of the *Acanthamoeba* genus in aquatic sources has been determined in different parts of Iran [13, 17, 22-25], but there is no information from Zanzan province. The present study was designed to detect the presence of *Acanthamoeba* genus in Zanzan province, northwest of Iran in the surface waters using a combined culture assay and the PCR method.

## 2. Materials and Methods

### 2.1. Sample collection

This descriptive cross-sectional survey was carried out in Zanzan province ( $36^{\circ}40' \text{N}$  and  $48^{\circ}29' \text{E}$ ), in northwest of Iran, between April 2015 and May 2016. Overall, 60 surface water samples

were collected from 30 various sources including the ponds in parks, the squares, the fountains in green fields dams and channels. From each sampling point, two water samples were placed in 500mL labeled and the sterile bottles transported immediately to the laboratory in the department of parasitology and Mycology, School of Medicine, Zanzan University of Medical Sciences, Zanzan, Iran, for further procedures.

### 2.2. Isolation and Cultivation

Each sample was filtrated through a  $0.45\mu\text{m}$  pore-size cellulose nitrate membranes using a vacuum pump [26]. After that, the filters were put on to the 1.5% non-nutrient agar (NNA) plates enriched with heat-killed *Escherichia coli* as a food source for the outgrowth of amoebae [27, 28]. The plates were incubated at the room temperature and monitored daily for up to 2 month in order to evaluate the growth of *Acanthamoeba* using an inverted microscope [29]. Then all the positive cultures were cloned to obtain a single cell line as well as to eliminate the fungal and bacterial contamination [30].

### 2.3. The Morphological identification

The preparations were tested for the presence of trophozoites and the cysts using the Giemsa staining assay [31] according to pages of taxonomy keys [29].

### 2.4. The DNA extraction

The growing amoebae were harvested from the surface of culture plates, washed with the sterile PBS, and centrifuged at 1000 rpm for 15mins. The DNA extraction was performed using the QIA amp DNA mini kit (Qiagene GmbH, Germany) according to the manufacturer's instructions. Then, the DNA was kept at  $-20^{\circ}\text{C}$  until the amplification of polymerase chain reaction (PCR).

### 2.5. PCR

The PCR procedure was carried out by amplifying a 423bp to 551bp region of the 18S rRNA gene (Rns) defined as ASA. The S1 that includes the hyper variable diagnostic fragments 3

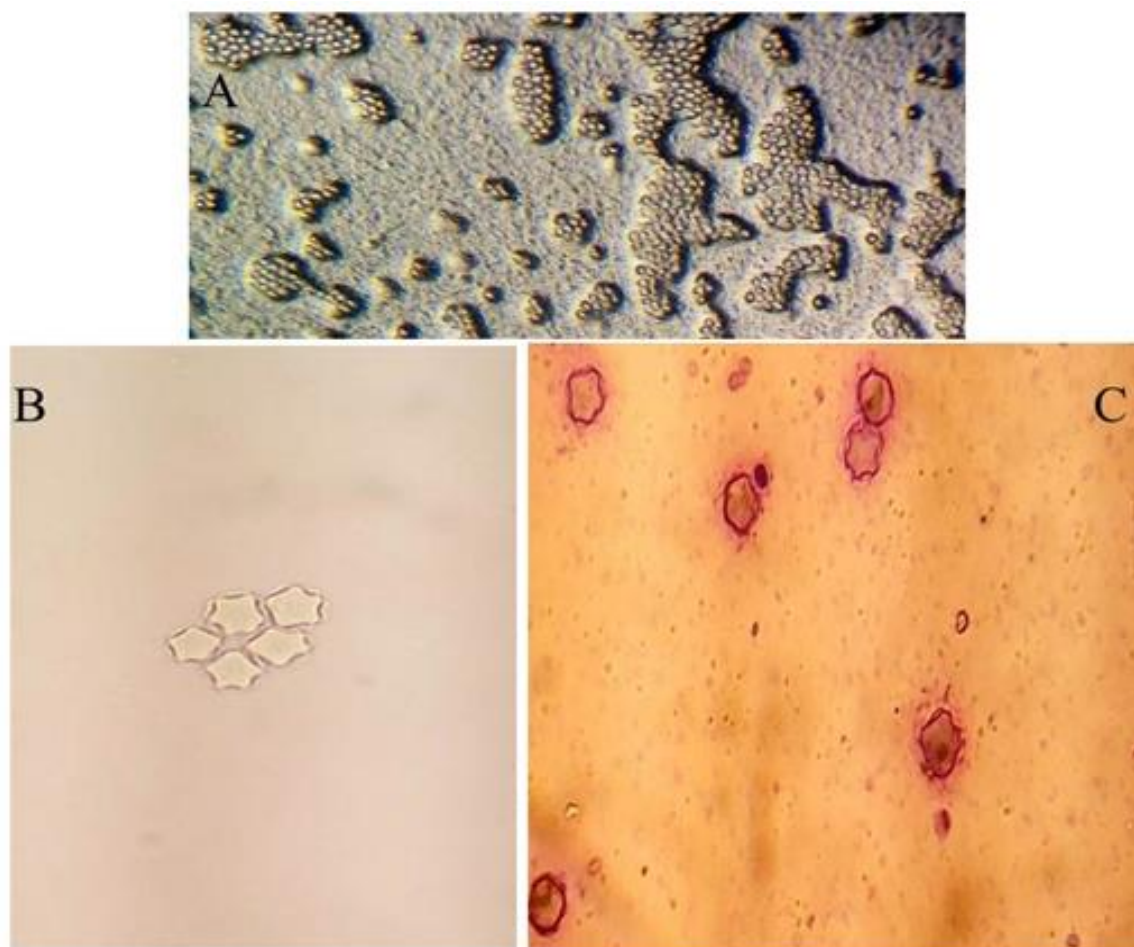
(DF3) using the genus of specific primers JDP1 (5'- GGCCAGATCGTTTACCGTGAA-3') and JDP2 (5'- TCTCACAAGCTGCTAGGGGAGTCA-3') [32].

The amplifications were performed in a final volume of 50 $\mu$ L containing 25 $\mu$ L Taq DNA Polymerase Master Mix Red (Ampliqon, Denmark), 5  $\mu$ L template DNA, 0.1 $\mu$ M of each primers and 17.5  $\mu$ L distilled water. The thermal cycling (Corbet research, Australia) conditions began with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturing for 30 seconds at 94°C, annealing for 45 seconds at 56°C, the extension took for 30 seconds at 72°C, and the final elongation was done for 10 min at 72°C. The PCR products were electrophoresed on a 2% agarose gel, and then stained with ethidium bromide and visualized under a UV Trans illuminator.

### 3. Results and Discussion

#### 3.1. Morphological identification

The recognition of *Acanthamoeba* at the genus level in the survey was based on the double walled cysts (Fig. 1) and the flattened trophozoites with acanthopodia, spine-like pseudopodia, on their surfaces (Fig.2). Out of 60 water samples collected in different recreational water sources, 30 (50%) were positive for *Acanthamoeba* spp based on the morphological criteria in non-nutrient agar (NNA) culture. The dams were the water sources with the highest number of positive samples, displaying 10/12 (83%) of positive culture isolates. A lower rate of positivity with 2/14 (14.3%) was obtained from channels (Table 1).



**Fig. 1:** Light micrographs of *Acanthamoeba* spp. cysts obtained from water samples.

A: Cysts on NNA (10X), B: Cysts in saline (40X), C: Cysts in Giemsa stain (40X).



**Fig. 2:** *Acanthamoeba* trophozoites in saline (40X).

**Table 1:** The Frequency of *Acanthamoeba* spp. in various water sources in Zanzan province, Iran.

Water sources	No. of examined samples	Positive Culture samples (%)
Park	10	5(50%)
Square and fountain	24	13 (54.2%)
Dam	12	10 (83.32%)
Channel	14	2 (14.3%)
Total	60	30 (50%)

### 3.2. The Molecular identification

The cultured positive samples of *Acanthamoeba* isolates, were further confirmed by the polymerase chain reaction (PCR) method, which were done by amplifying a portion of the 18S rRNA gene applying the genus specific primers pairs, The JDP1 and JDP2, and a nearly 500bp band detected on the agarose gel for all the positive isolates (Fig. 3).

The genus of *Acanthamoeba*, is the etiological agent of granulomatous amoebic encephalitis

(GAE), The *Acanthamoeba* keratitis (AK), and the disseminated tissue infections, are the ubiquitous protozoan parasite which have been widely found in the various water sources such as lakes, pools, tap water, thermal water, bottled mineral waters, cooling water, sea water, treated water, aquarium, rain water, wastewater and recreational waters [17, 33-36].

The food availability for the growth of *Acanthamoeba* spp and the resistance of the cyst stage of these amoebae to chlorination, ozonation and filtration by biofilm formation, probably creates the potential for high distribution of *Acanthamoeba* spp in the aquatic environments (25, 37-39). Thus, this alarmingly frequency of this amoeba in water resources represents a significant hazard for the high risk populations, including the contact lens wearers and immunocompromised individuals [13, 21, 26].

There are a few reports available regarding the presence of *Acanthamoeba* spp in different water sources in Iran [12, 13, 15, 22, 24, 40]. The results of previous studies in Iran showed that an increasing rate, in addition to being in the *Acanthodea* keratitis, was also in an undistinguished encephalitis in the immunocompromised patients [5, 19, 20, 22].

Accordingly, the isolation and identification of *Acanthamoeba* spp in water sources may play an important role in the prevention and control of the above mentioned serious problems. This is the first comprehensive research representing the occurrence of *Acanthamoeba* spp, using the morphological and molecular methods, in Zanzan province, Iran. This province is located in North-West of Iran bordering the provinces of East Azerbaijan, West Azerbaijan, Hamedan, Kordestan, Gilan, Ghazvin and Ardabil. Zanzan covers a region of 21.773km<sup>2</sup> where there are a lot of water sources such as dams, rivers, mineral water springs and green areas with superficial waters which are a hygienic risk for the populations who use these kinds of water in their life. In this survey, based on the morphological assessment, about half of the water samples were identified as the *Acanthamoeba* spp. Also, all of

the positive samples were reconfirmed by the molecular method as *Acanthamoeba* spp, the obtained results in the present study give further evidence of the presence of *Acanthamoeba* spp in the surface waters exist in Iran which is in accordance with other investigations worldwide [6, 15, 17, 22, 41-43].

The majority of the studies which studied the water samples were associated with the human recreational activity .

Therefore, the water sources in Zanzan province could be considered a significant public health hazard. The rate of contamination in Zanzan province was lower than it is in other studies which have been carried out in worldwide such as Khyber Pakhtunkhwa/Pakistan (92%), Northern parts/Poland (65%), Mazandaran/Iran (85%), Kish Island/Iran (66.7%), Bojnurd/Iran (68%), Ahvaz/Iran (71.6%) and Tehran/Iran (80%) [12, 13, 25, 40, 44-46]. The level of water contamination detected in this research was higher than the reports from Central and Southern parts/Italy (28.7%), Kayseri/Turkey (6.5%),

Sivas/Turkey (4.4%), Leon/Nicaragua (21%), Birjand/Iran (38%), East Azerbaijan/Iran (25.4%), Gilan/Iran (30%), Shiraz/Iran (32.5%) and Tehran/Iran (32%) [5, 24, 36, 47-52]. Overall, the high variability observed in the frequency rates in different localities could be related to several parameters, including the geographical factors, the climatic conditions, the seasonal changes, the water type, the samples of collection, the methodology of study and the diagnostic techniques [9, 40, 52, 53]. In the present work, the highest distribution of *Acanthamoeba* spp were found in dams and water sources, while the lowest frequency was recorded in the channels of water resources. The discrepancy of contamination rate in various kinds of aquatic environments may be due to the number of water samples examined, the water exposure through air, soil and feces, the flow and circulation of waters, water speed, the rate of water temperature , the biotic and abiotic factors, the existence of phagocytosing microorganisms and water treatment condition.

Thus, the comparison of all such investigations is not logical [15, 17, 38, 40].



**Fig. 3:** The Gel electrophoresis of the PCR products of *Acanthamoeba* spp. isolated from the water samples of Zanzan province, Iran. Lane M: standard DNA marker (250 bp), lane NC: negative control, lane PC: positive control and the lanes 1 to 9 are representative of the water *Acanthamoeba* isolates.

## 4. Conclusion

Although, the present survey gives the baseline information about water contamination with *Acanthamoeba* spp and will provide important knowledge regarding that water could be a significant transmission agent for *Acanthamoeb* spp, it requires further studies to focus on the determination of *Acanthamoeba* genotypes in the environmental samples and human in order to develop the proper methods for prevention and control of the severe and fatal complications.

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## References

1. Risler A, Coupat-Goutaland B, Pélandakis M. Genotyping and Phylogenetic Analysis of *Acanthamoeba* Isolates Associated with Keratitis. *J Parasitol Res.* 2013; 112: 3807-9.
2. Di Cave D, D'Alfonso R, Comlavi KAD, D'Orazi C, Monno R, Berrilli F. Genotypic Heterogeneity Based on 18S-rRNA Gene Sequences among *Acanthamoeba* Isolates from Clinical Samples in Italy. *Exp Parasitol.* 2014; 145: 46- 49.
3. Rocha-Cabrera P, Reyes-Batlle M, Martín-Navarro CM, Dorta-Gorrín A, López-Arencibia A, Sifaoui I, et al. Detection of *Acanthamoeba* on the Ocular Surface in a Spanish Population Using the Schirmer Strip Test: Pathogenic Potential, Molecular Classification and Evaluation of the Sensitivity to Chlorhexidine and Voriconazole of the Isolated *Acanthamoeba* Strains. *J Med Microbiol.* 2015; 64: 849- 4.
4. Santos Gomes T, Magnet A, Izquierdo F, Vaccaro L, Redondo F, Bueno S, et al. *Acanthamoeba* spp. in Contact Lenses from Healthy Individuals from Madrid, Spain. *PLoS One.* 2016; 11: e0154246.
5. Nazar M, Haghighi A, Niyyati M, Eftekhar M, Tahvildar-Biderouni F, Taghipour N, et al. Genotyping of *Acanthamoeba* Isolated from Water in Recreational Areas of Tehran, Iran. *J Water Health.* 2011; 9: 603-5.
6. Khezri A, Fallah E, Mostafazadeh M, Spotin A, Shahbazi A, Mahami-Oskouei M, et al. Molecular and Morphometric Characterization of *Acanthamoeba* spp. from Different Water Sources of Northwest Iran as a Neglected Focus, Co-Bordered With the Country of Iraq. *Jundishapur J Microbiol.* 2016; 9: e38481.
7. Khan NA. *Acanthamoeba*: Biology and Increasing Importance in Human Health. *FEMS Microbiol Rev.* 2006; 30: 564-31.
8. Hajjalilo E, Behnia M, Tarighi F, Niyyati M, Rezaeian M. Isolation and Genotyping of *Acanthamoeba* Strains (T4, T9, and T11) from Amoebic Keratitis Patients in Iran. *J Parasitol Res.* 2016; 115: 3147- 4.
9. Al-Herrawy A, Heshmat M, Abu Kabsha S, Gad M, Lotfy W. Occurrence of *Acanthamoeba* Species in the Damanhour Drinking Water Treatment Plant, Behera Governorate (Egypt). *Reports Parasitol.* 2015; 4: 15-21.
10. Shin HJ, Im KI. Pathogenic Free-Living Amoebae in Korea. *Korean J Parasitol.* 2004; 42: 93-119.
11. Mirjalali H, Niyyati M, Abedkhozasteh H, Babaei Z, Sharifdini M, Rezaeian M. Pathogenic Assays of *Acanthamoeba* Belonging to the t4 Genotype. *Iran J Parasitol.* 2013; 8: 530.
12. Rahdar M, Niyyati M, Salehi M, Fegghi M, Makvandi M, Pourmehdi M, et al. Isolation and Genotyping of *Acanthamoeba* Strains from Environmental Sources in Ahvaz City, Khuzestan



- Province, Southern Iran. *Iran J Parasitol.* 2012; 7: 22-26.
13. Mahmoudi MR, Rahmati B, Seyedpour SH, Karanis P. Occurrence and Molecular Characterization of Free-living Amoeba Species (*Acanthamoeba*, *Hartmannella*, and *Saccamoeba limax*) in Various Surface Water Resources of Iran. *J Parasitol Res.* 2015; 114: 4669-5.
14. Le Calvez T, Trouilhé MC, Humeau P, Moletta-Denat M, Frère J, Héchard Y. Detection of Free-living Amoebae by Using Multiplex Quantitative PCR. *Mol Cell Probes.* 2012; 26: 116-120.
15. Manesh RM, Niyiyati M, Yousefi HA, Eskandarian AA. Isolation of *Acanthamoeba* spp. from Different Water Sources in Isfahan, Central Iran, 2014. *J Parasit Dis.* 2016; 40: 1483-3.
16. Coşkun KA, Özçelik S, Tutar L, Elaldı N, Tutar Y. Isolation and Identification of Free-Living Amoebae from Tap Water in Sivas, Turkey. *Biomed Res Int.* 2013; 2013: 675145.
17. Niyiyati M, Saberi R, Latifi A, Lasjerdi Z. Distribution of *Acanthamoeba* Genotypes Isolated from Recreational and Therapeutic Geothermal Water Sources in Southwestern Iran. *Environ Health Insights.* 2016; 10: 69-74.
18. Paterson GN, Rittig M, Siddiqui R, Khan NA. Is *Acanthamoeba* Pathogenicity Associated with Intracellular Bacteria? *Exp parasitol.* 2011; 129: 207-210.
19. Niyiyati M, Rezaeian M. Current Status of *Acanthamoeba* in Iran: A Narrative Review Article. *Iran J Parasitol.* 2015; 10: 157-163.
20. Memari F, Niyiyati M, Haghighi A, Tabaei SJS, Lasjerdi Z. Occurrence of Pathogenic *Acanthamoeba* Genotypes in Nasal Swabs of Cancer Patients in Iran. *J Parasitol Res.* 2015; 114: 1907-5.
21. Gianinazzi C, Schild M, Zumkehr B, Wüthrich F, Nüesch I, Ryter R, et al. Screening of Swiss Hot Spring Resorts for Potentially Pathogenic Free-Living Amoebae. *Exp Parasitol.* 2010; 126: 45-53.
22. Armand B, Motazedian M, Asgari Q. Isolation and Identification of Pathogenic Free-Living Amoeba from Surface and Tap Water of Shiraz City Using Morphological and Molecular Methods. *J Parasitol Res.* 2016; 115: 63-68.
23. Badirzadeh A, Niyiyati M, Babaei Z, Amini H, Badirzadeh H, Rezaeian M. Isolation of Free-Living Amoebae from Sarein Hot Springs in Ardebil Province, Iran. *Iran J Parasitol.* 2011; 6: 1-8.
24. Behniafar H, Niyiyati M, Lasjerdi Z. Molecular Characterization of Pathogenic *Acanthamoeba* Isolated from Drinking and Recreational Water in East Azerbaijan, Northwest Iran. *Environ Health Insights.* 2015; 9: 7-12.
25. Niyiyati M, Lasgerdi Z, Lorenzo-Morales J. Detection and Molecular Characterization of Potentially Pathogenic Free-Living Amoebae from Water Sources in Kish Island, Southern Iran. *Microbiol Insights.* 2015; 1: 1-6.
26. Rezaeian M, Niyiyati M, Farnia S, Haghi AM. Isolation of *Acanthamoeba* spp. from Different Environmental Sources. *Iran J Parasitol.* 2008; 3: 44-47.
27. Khan NA. *Acanthamoeba: Biology and Pathogenesis.* Horizon Scientific Press; 2009.
28. Rezaeian M, Farnia S, Niyiyati M, Rahimi F. Amoebic Keratitis in Iran (1997-2007). *Iran J Parasitol.* 2007; 2: 1-6.
29. Page FC. A New Key to Freshwater and Soil Gymnamoebae: with Instructions for Culture. *FC Page: Freshwater Biological Association.* 1988.
30. Chan L-L, Mak J-W, Low Y-T, Koh T-T, Ithoi I, Mohamed SM. Isolation and Characterization of *Acanthamoeba* spp. from Air-Conditioners in Kuala Lumpur, Malaysia. *Acta Trop.* 2011; 117: 23-30.

31. Garcia LS. Diagnostic Medical Parasitology: American Society for Microbiology Press; 2006.
32. Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV, Markus MB, et al. Use of Subgenetic 18S Ribosomal DNA PCR and Sequencing for Genus and Genotype Identification of *Acanthamoebae* from Humans with Keratitis and from Sewage Sludge. *J Clin Microbiol.* 2001; 39: 1903-8.
33. Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as Agents of Disease in Humans. *Clin Microbiol Rev.* 2003; 16: 273-307.
34. Bagheri H, Shafiei R, Shafiei F, Sajjadi S. Isolation of *Acanthamoeba* Spp. from Drinking Waters in Several Hospitals of Iran. *Iran J Parasitol.* 2010; 5: 19-25.
35. Gatti S, Rama P, Matuska S, Berrilli F, Cavallero A, Carletti S, et al. Isolation and Genotyping of *Acanthamoeba* Strains from Corneal Infections in Italy. *J Med Microbiol.* 2010; 59: 1324-6.
36. Kuk S, Yazar S, Dogan S, Çetinkaya Ü, Şakalar Ç. Molecular Characterization of *Acanthamoeba* Isolated from Kayseri Well Water. *Turk J Med Sci.* 2013; 43: 12-17.
37. Edagawa A, Kimura A, Kawabuchi-Kurata T, Kusuhara Y, Karanis P. Isolation and Genotyping of Potentially Pathogenic *Acanthamoeba* and *Naegleria* Species from Tap-Water Sources in Osaka, Japan. *Parasitol Res.* 2009; 105: 1109-8.
38. Adamska M, Leonska-Duniec A, Lanocha N, Skotarczak B. Thermophilic Potentially Pathogenic Amoebae Isolated from Natural Water Bodies in Poland and Their Molecular Characterization. *Acta Parasitol.* 2014; 59: 433-8.
39. Astorga B, Lorenzo-Morales J, Martín-Navarro CM, Alarcón V, Moreno J, González AC, et al. *Acanthamoeba* Belonging to T3, T4, and T11: Genotypes Isolated from Air-Conditioning Units in Santiago, Chile. *J Eukaryot Microbio.* 2011; 58: 542-2.
40. Niyiyati M, Lasjerdi Z, Nazar M, Haghghi A, Mojarad EN. Screening of Recreational Areas of Rivers for Potentially Pathogenic Free-Living Amoebae in the Suburbs of Tehran, Iran. *J Water Health.* 2012; 10: 140-6.
41. Hooshyar H, Hosseinbigi B, Saraei M, Alizadeh S, Eftakhar M, Rasti S, et al. Genotyping of *Acanthamoeba* Isolated from Surface and Stagnant Waters of Qazvin, Central Iran. *Iran Red Crescent Med J.* 2013; 15: 536-538.
42. Lorenzo-Morales J, Ortega-Rivas A, Foronda P, Martínez E, Valladares B. Isolation and Identification of Pathogenic *Acanthamoeba* Strains in Tenerife, Canary Islands, Spain from Water Sources. *Parasitol Res.* 2005; 95: 273-277.
43. Todd CD, Reyes-Batlle M, Piñero JE, Martínez-Carretero E, Valladares B, Streete D, et al. Isolation and Molecular Characterization of *Acanthamoeba* Genotypes in Recreational and Domestic Water Sources from Jamaica, West Indies. *J Water Health.* 2015; 13: 909-10.
44. Tanveer T, Hameed A, Muazzam AG, Jung SY, Gul A, Matin A. Isolation and Molecular Characterization of Potentially Pathogenic *Acanthamoeba* Genotypes from Diverse Water Resources Including Household Drinking Water from Khyber Pakhtunkhwa, Pakistan. *Parasitol Res.* 2013; 112: 2925-7.
45. Salehi M. *Acanthamoeba* Strains Genotypes Prevalence in Water Sources in Bojnurd City: Short Communication. *J Birjand Univ Med Sci.* 2014; 21: 260-6.
46. Lass A, Szostakowska B, Idzińska A, Chomicz L. The First Genotype Determination of *Acanthamoeba* Potential Threat to Human Health, Isolated from Natural Water Reservoirs in Poland. *Parasitol Res.* 2014; 113: 2693-6.
47. Niyiyati M, Nazar M, Haghghi A, Nazemalhosseini E. Reporting of T4 Genotype of *Acanthamoeba* Isolates in Recreational Water Sources of Gilan Province, Northern Iran. *Novel Biomed.* 2015; 3: 20-24.



48. Ghader-ghadr Sh, Solhjoo K, Norouz-nejad MJ, Rohi R, Zia-Jahromi S. Isolation and Identification of Free Living Amoeba (*Naegleria* and *Acanthamoeba*) in Shiraz Water Resources by Morphological Criteria. *J Jahrom Univ Med Sci*. 2012; 10: 26-33.
49. Leiva B, Clasdatter E, Linder E, Winiecka-Krusnell J. Free-Living *Acanthamoeba* and *Naegleria* spp. Amebae in Water Sources of León, Nicaragua. *Rev Biol Trop*. 2008; 56: 439-446.
50. Ozçelik S, Coskun KA, Duzlu O, Ahmet A, Malatyali E. The Prevalence, Isolation and Morphotyping of Potentially Pathogenic Free-Living Amoebae from Tap Water and Environmental Water Sources in Sivas. *Turkiye Parazitol Derg*. 2012; 36: 198-203.
51. Behravan M, Behniafar H, Einipour S, Dorani N, Naghizadeh A. Morphological and Molecular Identification of *Acanthamoeba* Spp from Surface Waters in Birjand, Iran, During 2014-2015. *Arch Hyge Sci*. 2016; 5: 117-122.
52. Di Filippo MM, Santoro M, Lovreglio P, Monno R, Capolongo C, Calia C, et al. Isolation and Molecular Characterization of Free-Living Amoebae from Different Water Sources in Italy. *Int J Environl Res Publ Health*. 2015; 12: 3417-10.
53. Pezeshki A, Haniloo A, Alejafar A, Mohammadighalehbin B. Detection of *Toxocara* spp. Eggs in the Soil of Public Places in and Around of Ardabil City, Northwestern Iran. *Iran J Parasitol*. 2017; 12: 136-142.