Turkish Journal of Medical Sciences

Volume 43 | Number 1 Article 3

1-1-2013

Molecular characterization of Acanthamoeba isolated from Kayseri well water

SALİH KUK

SÜLEYMAN YAZAR

SÜHEYLA DOĞAN

ÜLFET ÇETİNKAYA

ÇAĞRI ŞAKALAR

Follow this and additional works at: https://journals.tubitak.gov.tr/medical

Part of the Medical Sciences Commons

Recommended Citation

KUK, SALİH; YAZAR, SÜLEYMAN; DOĞAN, SÜHEYLA; ÇETİNKAYA, ÜLFET; and ŞAKALAR, ÇAĞRI (2013) "Molecular characterization of Acanthamoeba isolated from Kayseri well water," *Turkish Journal of Medical Sciences*: Vol. 43: No. 1, Article 3. https://doi.org/10.3906/sag-1205-26
Available at: https://journals.tubitak.gov.tr/medical/vol43/iss1/3

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Medical Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2013) 43: 12-17
© TÜBİTAK
doi:10.3906/sag-1205-26

Molecular characterization of *Acanthamoeba* isolated from Kayseri well water

Salih KUK[1], Süleyman YAZAR[1,*], Süheyla DOĞAN[1], Ülfet ÇETİNKAYA[1], Çağrı SAKALAR[2]
[1]Department of Parasitology, Faculty of Medicine, Erciyes University, Kayseri, Turkey
[2]Halil Bayraktar Vocational High School, Erciyes University, Kayseri, Turkey

Received: 10.05.2012 • **Accepted:** 10.06.2012 • **Published Online:** 18.01.2013 • **Printed:** 18.02.2013

Aim: Potentially pathogenic free-living amoebae have a cosmopolitan distribution in soil, dust, air, and water. Generally, environmental free-living amoebae do not threaten human health. This study aimed to investigate the presence of *Acanthamoeba* in well waters drawn from different locations in Kayseri, Turkey, which are mainly used for drinking or irrigation by the residents in the region.

Materials and methods: Twenty-nine samples, including 26 well water sediment samples and 3 tap water samples as the control, were collected from 3 different locations. Melting snow feeds 5, rain water 18, and tap water 3 of these wells.

Results: Five of 26 well water samples were 19.23% positive for *Acanthamoeba* by both PCR and agar culture. All of these *Acanthamoeba* were characterized as the T4 genotype group.

Conclusion: This is the first report from Turkey on the isolation and identification of *Acanthamoeba*. Further studies with a wide series are warranted, focusing on in vitro cytotoxicity and in vivo pathogenicity of isolates.

Key words: *Acanthamoeba*, well water, genotype, Kayseri, Turkey

1. Introduction

Parasitic diseases are widespread and dangerous in humans and animals worldwide (1–3). Potentially pathogenic free-living amoebae (FLA) have worldwide distribution in water, soil, dust, and air. They have been isolated from lakes, pools, swimming and therapeutic pools, tap water, thermal water, cooling water, bottled mineral waters, hot tubs, air-conditioning units, and eyewash solutions (4–8). Infections due to pathogenic FLA in these environments and items have been largely documented. People may inevitably come into contact with potentially pathogenic FLA due to this worldwide distribution as has already been evidenced by antibody titers in human populations (9,10).

In general, environmental FLA do not threaten humans. However, FLA such as *Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Sappinia pedata* are known to be opportunistic parasites that can lead to severe pathologies (11–13). Central nervous system (CNS) infections caused by FLA include primary amoebic meningoencephalitis with *N. fowleri* and granulomatous amoebic encephalitis, which is due to infections with several *Acanthamoeba* as well as *B. mandrillaris*, mainly in immunocompromised humans and in animals. In addition, *Acanthamoeba* and *B. mandrillaris* are known to cause skin infections, but it is also important to emphasize that *Acanthamoeba* spp. also cause keratitis, which may further result in blindness (12,14).

Therefore, the interest in pathogenic FLA, epidemiology of FLA, and infections associated with FLA is gradually increasing. To the best of our knowledge, this study will be the first report from Kayseri investigating *Acanthamoeba* in well water.

2. Materials and methods

2.1. Sampling

Samples were obtained from well water sediment in the town of Hacılar in the foothills of Erciyes Mountain between March and May 2011. The wells are also known as snow wells and are 2 and 3 m deep and square-shaped. This well water is currently used for drinking and irrigation. The altitudes of Erciyes Mountain and Hacılar are 3917 m and 1350 m from sea level, respectively. Twenty-nine water samples, including 26 well and 3 tap waters, were collected from 3 different locations as presented in the Table. Plastic tubes of 50 mL were used to collect water and were pelleted for 10 min at 1000 rpm. The pellets were resuspended in 100 µL of supernatant and used.

* Correspondence: syazar@erciyes.edu.tr

2.2. Isolation and culturing of trophozoites

Resuspended pellets were gently pipetted onto a nonnutrient agar plate (1.5% agar in Page's saline) and were allowed to adsorb and dry. Subsequently, the plates were sealed with Parafilm and incubated upside down at 37 °C and additionally at 42 °C. A daily inspection of plates was done by light microscopy until morphological structures suggestive of amoeba trophozoites were detected. Cultures lacking morphological features of amoebae within 15 days were considered as negative and discarded.

2.3. Characterization of the isolates

The trophozoites were gently scraped from an agar plate using a sterile pipette, resuspended in phosphate buffered saline, and pelleted at 1000 rpm for 15 min. DNA was extracted from the pellet using the QIAamp DNA Mini Kit (QIAGEN, USA) according to the manufacturer's instructions. The genomic DNA was subjected either to polymerase chain reaction (PCR) yielding the specific recognition of *Acanthamoeba* spp. (Nelson-PCR, JDP-PCR), or to a pan-PCR recognizing FLA in general (FLA-PCR). The following primer pairs were used for PCRs: FLA-PCR: P-FLA-F (5'-CGCGGTAATTCCAGCTCCAATAGC-3'); P-FLA-R: (5'-CAGGTTAAGGTCTCGTTCGTTAAC-3'); target: 18S rDNA; Nelson PCR: NelsonF (5'-GTTTGAGGCAATAACAGGT-3'); NelsonR (5'-GAATTCCTCGTTGAAGAT-3'); target: 18S rDNA; JDP PCR: JDP1 (5'-GGCCCAGATCGTTTACCGTGAA-3'); JDP2 (5'-TCTCACAAGCTGCTAGGGAGTCA-3'); target: 18S rDNA (8,15,16). The PCRs were carried out in a 25-µL volume containing 12.5 µL of 2X PCR Master Mix (Vivantis, Malaysia), 2 µL of 20 pmol of each primer, and 1 µL of genomic template DNA, and they were performed in a PCR labcycler (SENSQUEST, Germany). Products from all PCRs were visualized on 2% agarose gel containing ethidium bromide.

2.4. Phylogenetic analysis

For sequence analyses, PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's instructions. The purified products of JDP-PCR of *Acanthamoeba* were subjected to cycle sequencing by the dideoxynucleotide chain termination method using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham), with JDPF used as a sequencing primer. The products were separated in an automated DNA genetic analyzer (MegaBACE 500 Genetic Analyzer). The GenBank BLAST program was used for comparisons. A sequence of 423–551 bp was read for each isolate. These were aligned with the corresponding sequences from 18 reference *Acanthamoeba* isolates, including genotypes T1–T15 under the following accession numbers: T1: *A. castellanii* CDC:0981:V006 (U07400); T2: *A. palestinensis* Reich; ATCC 30870 (U07411); T3: *A. griffini* S-7 ATCC 30731 (U07412); T4: *A. castellanii* Ma ATCC 50370 (U07414); T4: *Acanthamoeba* sp. S27 (DQ087323); T4: *Acanthamoeba* sp. ACA1 (EF654665); T4: *Acanthamoeba* sp. ACA7 (EU741257); T4: *Acanthamoeba* sp. ACA9 (EU741252); T5: *A. lenticulata* PD2S (U94741); T6: *A. palestinensis* 2802 (AF019063); T7: *A. astronyxis* CCAP 1534/1 (AF239293); T8: *A. tubiashi* OC-15C (AF019065); T9: *A. comandoni* Comandon & de Fonbrune (AF019066); T10: *A. culbertsoni* Lilly A-1 (AF019067); T11: *Acanthamoeba* sp. PN14 (AF333608); T12: *A. healyi* CDC 1283:V013 (AF019070); T13: *Acanthamoeba* sp. UWC9 (AF132134); and T15: *A. jacobsi* AC305 (AY262365); T15: *Acanthamoeba* sp. ACA12 (FJ195367).

Three sequences of our *Acanthamoeba* isolates were used in phylogenetic tree construction. JDP sequences were entered in MEGA5 for construction of the phylogenetic trees using maximum parsimony (MP) and distance methods, namely neighbor-joining, an unweighted pair-group method with arithmetic averages (UPGMA), and minimum evolution (17). Branch support was given using 1000 bootstrap replicates in MEGA5 (17).

3. Results

3.1. Isolation and culture of trophozoites

Sources, sample ID, purpose and temperature of water, and positive samples are shown in the Table. Five water samples (OV1, HM3, HM4, SF2, and SF3) from 26 different samples were positive for *Acanthamoeba* (19.2%) after in vitro culture at 37 °C for 15 days (Table). One sample from a well fed by snow water and 4 samples from wells fed by rain water were positive for FLA. Samples originating from 3 different tap waters were negative (OV10, HM10, and SF9) (Table). The amoebae have characteristic spine-like pseudopodia called acanthapodia on the surface of trophozoites, and double-walled cysts. Therefore, isolates OV1, HM3, HM4, SF2, and SF3 were identified as belonging to the genus *Acanthamoeba*.

3.2. Molecular identification of the isolates and phylogenetic analysis

Five samples scored positive in FLA-PCR while all samples were also positive in both Nelson-PCR and JDP-PCR. We observed an expected amplicon of JDP-PCR in 5 well water sediments (Figure 1). Three different *Acanthamoeba* isolates were obtained from sequence analyzing of the JDP-PCR. While the sequence of JDP DNA of the HM3 isolate was the same as the sequence of HM4, the sequence of JDP DNA of the SF2 isolate was the same as the sequence of SF3. OV1, HM3, and SF2 were named respectively as ERC-B1, ERC-B2, and ERC-B3. Sequences of 3 isolates in the present study were deposited in the GenBank database and are available under accession numbers JN793396 (ERC-B1), JN793397 (ERC-B2), and JN793398 (ERC-B3).

Acanthamoeba isolates (OV1, HM3, and SF2) were further investigated by phylogenetic analyses, which were

Table. Water samples investigated for free-living amoebae.

Hacılar region	ID of samples	Water content	Temperature[a]	Isolation[b]
Oren vineyard (OV)	OV1-ww	1	3.8 °C	*Acanthamoeba*
	OV2-ww	1	8.0 °C	-
	OV3-ww	2	6.5 °C	-
	OV4-ww	2	7.2 °C	-
	OV5-ww	2	0.8 °C	-
	OV6-ww	2	9.0 °C	-
	OV7-ww	2	9.1 °C	-
	OV8-ww	2	12.3 °C	-
	OV9-ww	3	7.1 °C	-
	OV10-tw	3	19.2 °C	-
Hasan Mountain (HM)	HM1-ww	1	16.2 °C	-
	HM2-ww	1	16.2 °C	-
	HM3-ww	2	17.7 °C	*Acanthamoeba*
	HM4-ww	2	15.1 °C	*Acanthamoeba*
	HM5-ww	2	13.3 °C	-
	HM6-ww	2	12.2 °C	-
	HM7-ww	2	14.4 °C	-
	HM8-ww	2	17.3 °C	-
	HM9-ww	3	11.1 °C	-
	HM10-tw	3	19.7 °C	-
Sakar Farm (SF)	SF1-ww	1	13.7 °C	-
	SF2-ww	2	14.9 °C	*Acanthamoeba*
	SF3-ww	2	11.6 °C	*Acanthamoeba*
	SF4-ww	2	14.7 °C	-
	SF5-ww	2	11.7 °C	-
	SF6-ww	2	11.9 °C	-
	SF7-ww	2	11.8 °C	-
	SF9-ww	2	11.6 °C	-
	SF8-ww	3	14.3 °C	-
	SF9-tw	3	21.6 °C	-

Samples: ww, well water; tw, tap water. Water content: 1, snow water; 2, rain water; 3, tap water.
[a]Water temperature at sampling.
[b]Dashes indicate water samples where no trophozoites were detected within 15 days of incubation, either due to the absence of trophozoites in the sample or due to the fact that the trophozoites were not able to grow at 37 °C.

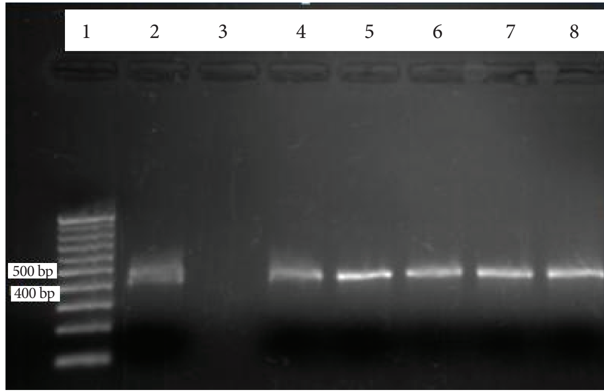

Figure 1. Agarose gel showing amplifications of PCR-JDP of *Acanthamoeba* species. Lane 1: 100-bp Plus DNA Ladder (Vivantis); lane 2: positive control, *Acanthamoeba castellanii*; lane 3: negative control; lanes 4–8: *Acanthamoeba* spp.-positive PCR product from obtained well water sediments (OV1, HM3, HM4, SF2, and SF3) in the town of Hacılar, Kayseri Province, Turkey.

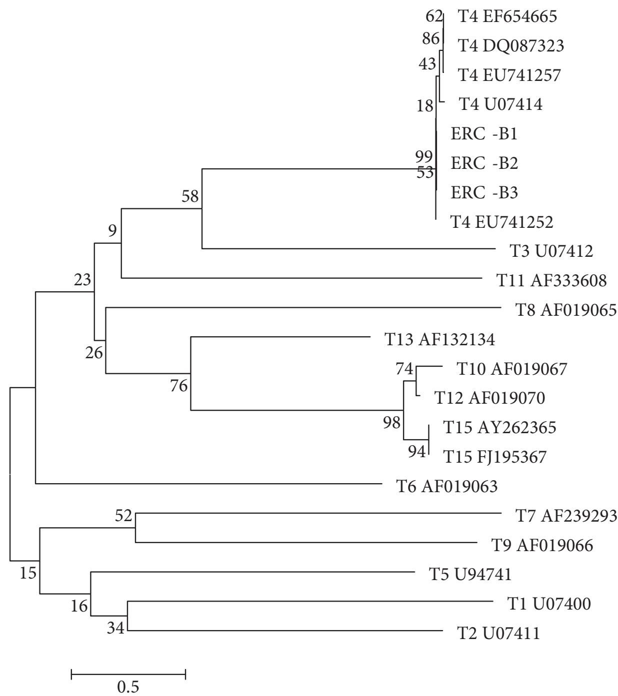

Figure 2. Neighbor-joining distance tree based on partial 18S rDNA sequences with 1000 bootstrap replications, produced in MEGA 5. The sequences from our isolates (GenBank accession nos. ERC-B1 (JN793396), ERC-B2 (JN793397), and ERC-B3 (JN793398)) were aligned with the corresponding sequences from 18 reference *Acanthamoeba* isolates, including genotype T1–T15 designations that correspond to sequences previously determined to be of that particular genotype, available in GenBank under the accession numbers reported in Materials and Methods. Bar index of dissimilarity is 0.5 among the different sequences.

done by sequencing of JDP-PCR amplimers and subsequent comparison of these sequences with corresponding regions from other known *Acanthamoeba* strains. The comparative investigation included all representative sequences available from GenBank and had previously been used for the definition of phylogenetic sequence types T1–T15 (18). Sequences from all 3 *Acanthamoeba* isolates obtained in the study were clustered in sequence type group T4 (Figure 2).

4. Discussion

The present study is the first report to investigate, isolate, and identify the *Acanthamoeba* that may exhibit a potential pathogenicity from selected Turkish well water sediments. We isolated *Acanthamoeba* in 19.2% of the investigated water samples. FLA are prevalent in soil and water. These environmental sites are known from other studies to potentially harbor pathogenic FLA including *Acanthamoeba* spp. and *N. fowleri*, which can lead to severe and lethal CNS infections (9). Therefore, screening of FLA in these sites is very important for human health. The rate of FLA was lower than in partially similar previous studies from the United States (143 of 330, 43.4%), Thailand (43 of 95, 45.2%), Japan (47 of 95, 49.5%), Osaka in Japan (257 of 375, 68.7%), and Bulgaria (31 of 35, 93. 9%) (4,8,19,20). Water samples were collected at 4 hot spring resorts in the temperature range of 33–40 °C, and 9 of the 31 water samples scored positive for FLA (29%) (7). The data of Gianinazzi et al. reported the presence of FLA in 75% of water samples investigated (6). In that study, temperature of samples ranged from 9.2 to 29.3 °C, but in ours it ranged from 0.8 to 19.7 °C. This discrepancy may be related to the low temperature of the wells in our region. Additionally, we did not investigate *Naegleria* in the well water of our region since the water temperatures were quite low (from 0.8 to 19.2 °C).

Acanthamoeba spp. are the most common amoebae, and probably the most common protozoa, to be found in soil and water samples, as evidenced by their presence at high percentages in samples taken in human-related aquatic habitats (21). The classification of the genus *Acanthamoeba* is still debated and molecular approaches have been used for precise identification of isolates. More than 24 named species have been described within the genus *Acanthamoeba*, including both pathogenic and nonpathogenic isolates. *Acanthamoeba* species were classified into 3 morphological groups due to alternating cyst appearance (22). The analysis of 18S rDNA of *Acanthamoeba* isolates has also been described, identifying 12 distinct sequence genotypes (T1–T12) and later adding genotypes T13, T14, and T15 (13,22–24). In our study, 4 of 26 well water sediments were positive for *Acanthamoeba* sp. A phylogenetic analysis of the 5 sequences showed that

2 sequences of the *Acanthamoeba* isolates were the same and 3 isolates were clustered into genotype T4.

In Turkey, a few studies have addressed the presence of potentially pathogenic FLA in environmental samples (25–27). Eighteen *Acanthamoeba* isolates have been isolated from 28 soil and 2 water samples. Ribosomal DNA sequencing revealed that 10 isolates belonged to the T2 genotype, 5 isolates belonged to T3, 2 isolates belonged to T4, and 1 isolate belonged to T7. These water samples were obtained from hot spring water (28).

The first study identifying *Acanthamoeba* keratitis in Turkey and the first isolation of genotype T9 in the country was performed by Ertabaklar et al. (29). The *Acanthamoeba* strain isolated from a corneal scraping was identified as genotype T4. Three more *Acanthamoeba* strains isolated from sites of possible human contact with *Acanthamoeba* in the same geographical region, including a lens storage case, tap water, and soil, were subjected to molecular biological identification. While the strain from tap water exhibited genotype T4, 2 other isolates were identified as genotype T9. Based on PCR-amplified 18S rRNA gene analysis, the first isolate was identified as *Acanthamoeba* genotype T4 and the second as *Paravahlkampfia* sp. from the patient in İzmir, Turkey (30). Despite these studies, a detailed study has not been conducted for screening of FLA in environmental waters in Turkey.

We have identified, for the first time, *Acanthamoeba* isolates from 26 well water sediments in Turkey. Further research is needed in a wide series of subjects focusing on in vitro cytotoxicity and in vivo pathogenicity of isolates and assessing the phylogenetic and pathogenic association in *Acanthamoeba* infections.

Acknowledgments
We thank Associate Professor Zübeyde Akın Polat for reference isolate. The work was supported by the Erciyes University Scientific Research Projects Unit, Turkey (Project No. TA-11-3314).

References

1. Karabulut A, Polat Y, Türk M, Işık Balcı Y. Evaluation of rubella, *Toxoplasma gondii*, and cytomegalovirus seroprevalences among pregnant women in Denizli province. Turk J Med Sci 2011; 41: 159–64.
2. Hatam Nahavandı K, Fallah E, Asgharzadeh M, Mirsamadı N, Mahdavıpour B. Glutamate dehydrogenase and triose-phosphate-isomerase coding genes for detection and genetic characterization of *Giardia lamblia* in human feces by PCR and PCR-RFLP. Turk J Med Sci 2011; 41: 283–9.
3. Usluca S, Aksoy U. Detection and genotyping of *Cryptosporidium* spp. in diarrheic stools by PCR/RFLP analyses. Turk J Med Sci 2011; 41: 1029–36.
4. Caumo K, Rott MB. *Acanthamoeba* T3, T4 and T5 in swimming-pool water from Southern Brazil. Acta Tropica 2011; 117: 233–5.
5. 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: 1109–17.
6. Gianinazzi C, Schild M, Wüthrich F, Ben Nouir N, Füchslin HP, Schürch N et al. Screening Swiss water bodies for potentially pathogenic free-living amoebae. Res Microbiol 2009; 160: 367–74.
7. 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.
8. Tsvetkova N, Schild M, Panaiotov S, Kurdova-Mintcheva R, Gottstein B, Walochnik J et al. The identification of free-living environmental isolates of amoebae from Bulgaria. Parasitol Res 2004; 92: 405–13.
9. Visvesvara GS, Maguire JH. Pathogenic and opportunistic free-living amebas. *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases. Philadelphia: Churchill Livingstone; 2006. p.1114–25.
10. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. Clin Microbiol Rev 2002; 15: 342–54.
11. Gelman BB, Rauf SJ, Nader R, Popov V, Bokowski J, Chaljub G et al. Amoebic encephalitis due to *Sappinia diploidea*. JAMA 2001; 285: 2450–1.
12. Martinez AJ, Visvesvara GS. Free-living amphizoic and opportunistic amebas. Brain Pathol 1997; 7: 583–98.
13. Stothard DR, Schroeder-Diedrich JM, Awwad MH, Gast RJ, Ledee DR, Rodriguez-Zaragoza S et al. The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. J Eukaryot Microbiol 1998; 45: 45–54.
14. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol 2007; 50: 1–26.
15. Boggild AK, Martin DS, Lee TY, Yu B, Low DE. Laboratory diagnosis of amoebic keratitis: comparison of four diagnostic methods for different types of clinical specimens. J Clin Microbiol 2009; 47: 1314–8.
16. Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV, Markus MB et al. Use of subgenic 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: 1903–11.

17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28: 2731–9.

18. Di Cave D, Monno R, Bottalico P, Guerriero S, D'Amelio S, D'Orazi C et al. *Acanthamoeba* T4 and T15 genotypes associated with keratitis infections in Italy. Eur J Clin Microbiol Infect Dis 2009; 28: 607–12.

19. Ettinger MR, Webb SR, Harris SA, McIninch SP, Garman G, Brown BL. Distribution of free-living amoebae in James River, Virginia, USA. Parasitol Res 2003; 89: 6–15.

20. Nacapunchai D, Kino H, Ruangsitticha C, Sriwichai P, Ishih A, Terada M. A brief survey of free-living amebae in Thailand and Hamamatsu District Japan. Southeast Asian J Trop Med Pub Health 2001; 32 (Suppl 2): 179–82.

21. Lorenzo-Morales J, Monteverde-Miranda CA, Jiménez C, Tejedor ML, Valladares B, Ortega-Rivas A. Evaluation of *Acanthamoeba* isolates from environmental sources in Tenerife, Canary Islands, Spain. Ann Agric Environ Med 2005; 12: 233–6.

22. Pussard M, Pons R. Morfologie de la paroi kystique et toxonomie du genre Acanthomoeba (Porotozoa, Amoebida). Protistologica 1977; 8: 557–98.

23. Gast RJ. Development of an *Acanthamoeba*-specific reverse dot-blot and the discovery of a new ribotype. J Eukaryot Microbiol 2001; 48: 609–15.

24. Hewett MK, Robinson BS, Monis PT, Saint CP. Identification of a new *Acanthamoeba* 18S rRNA gene sequence type corresponding to the species *Acanthamoeba jacobsi* Sawyer, Nerad and Visvesvara, 1992 (Lobosea: Acanthamoebidae). Acta Protozool 2003; 42: 325–9.

25. Akın Z. Studies on isolation, characterization and pathogenicity of free-living amoebae from soil and fresh-water specimens in Sivas. MSc thesis, Cumhuriyet University Health Sciences Institute, Sivas, Turkey, 2000.

26. Saygı G. Erzurum'da topraktan *Acanthamoeba* türünün soyutlanması. Türkiye Parazitol Derg 1979; 2: 109–14.

27. Saygı G, Akın Z. Sivas'ta toprak ve termal su örneklerinden *Acanthamoeba* ve *Naegleria* türlerinin soyutulması. Türkiye Parazitol Derg 2000: 24: 237–42.

28. Kilic A, Tanyuksel M, Sissons J, Jayasekera S, Khan N. Isolation of *Acanthamoeba* isolates belonging to T2, T3, T4, and T7 genotypes from environmental samples in Ankara, Turkey. Acta Parasitol 2004; 49: 246–52.

29. Ertabaklar H, Türk M, Dayanır V, Ertug S, Walochnik J. *Acanthamoeba* keratitis due to *Acanthamoeba* genotype T4 in a non-contact-lens wearer in Turkey. Parasitol Res 2007; 100: 241–6.

30. Özkoç S, Tuncay S, Delibaş SB, Akisu C, Ozbek Z, Durak I et al. Identification of *Acanthamoeba* genotype T4 and *Paravahlkampfia* spp. from two clinical samples. J Med Microbiol 2008; 57: 392–6.