ORIGINAL RESEARCH

Contamination of tap water sources to *Acanthamoeba* spp. in selected cities of Hormozgan province, Iran

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Abstract

Background
Free-living amoeba (FLA) such as *Acanthamoeba* are ubiquitous and amphizoic protozoan parasites. These parasites can cause severe human infections. Water resources are one of the important habitats for FLAs. Waters in tanks and wells can be a habitat for *Acanthamoeba* species, particularly in warm climates. The present study was conducted to evaluate the presence of *Acanthamoeba* species in tap water resources of selected cities of Hormozgan Province, Southern Iran.

Material and methods
In this cross sectional study, 50 water samples (approximately 500 mL) were collected from tap water resources (25 samples from tanks and 25 samples from wells) of Hormozgan province. The samples were filtered, cultured on 1.5% non-nutrient agar medium. After incubation at room temperature, cloning was performed. Investigation of *Acanthamoeba* species was performed using the microscopic method and page key.

Results
Out of 50 samples, 14 samples (28%) were found positive. Out of 25 examined well samples 9 samples (36%) were found contaminated with *Acanthamoeba* spp., while only 5 (20%) of 25 tank samples were found positive. All samples were cloned successfully.

Conclusion
The high occurrence of *Acanthamoeba* spp. in water resources of Hormozgan province could be as a hazard for public health of the region, and using effective disinfectants and accurate monitoring may be a good suggestion.

Keywords: *Acanthamoeba*, Water resources, Hormozgan province

Introduction

Free-living amoebae (FLA) including *Acanthamoeba* species are ubiquitous and amphizoic protists, which have a wide distribution in both of natural and artificial environments. The amoeba was isolated from various kind of resources such as water, soil, dust, animal, human, hospital environment, and contact lens solutions. *Acanthamoeba* spp. are the most common FLAs with worldwide distribution (1, 2). Moreover, among ubiquitous and parasitic protozoans, genus *Acanthamoeba* has the most frequency in various types of water (3-6). The high rate of *Acanthamoeba* specific antibodies (50-100%) in sera of healthy individuals confirms high frequency of exposure to this protozoan (7-9). There is two stage during the life cycle of *Acanthamoeba* spp., the cyst stage is a stage that the organism is resistant to harsh environmental conditions such as adverse temperature and pH, and exposed to disinfectants, and on the other hand trophozoite is the active stage and is seen when environmental condition is favorable for the protozoa (10, 11).

Keratitis, encephalitis and skin granulomatous are three severe and fatal diseases that caused by FLAs (12-14). The most common disease that caused by *Acanthamoeba* spp. is keratitis. Amoebic keratitis (AK) is a sight-threating disease with severe eye pain. As mentioned earlier, water resources are favorable habitat and an important source of infection for potentially pathogenic *Acanthamoeba* spp., thus contaminated water resources are a hazard for AK high-risk groups including contact lens wearers (especially soft lens wearers), eye surgery, eye trauma, and immunocompromised patients (13, 14). Granulomatous amoebic encephalitis (GAE) is another disease that can be caused by *Acanthamoeba* species. Unlike AK, GAE is rare and fatal. Treatment of both diseases caused by this parasite is difficult and in most cases fails. On the other hand, the amoeba can carry pathogenic bacteria such as *Legionella*, *Pseudomonas* and *Helicobacter*, fungi, and viruses (13, 15, 16).

In Iran, FLAs especially *Acanthamoeba* spp. reported from different regions and various resources. However, there are limited studies on the occurrence of *Acanthamoeba* in warm regions. A previous study in Kish island showed a high presence of *Acathamoeba* in tap water sources (17).

The present study aimed to isolate and identify *Acanthamoeba* spp. from various water resources of Hormozgan province using culture method and microscopic examination.

Material and Methods

Study area

This study was conducted in Hormozgan province, Iran. The province is located in the south of the country (fig. 1). The province is a major trade center between Dubai, UAE and Iran using port of Bandar Abbas. Hormozgan province is limited by the Persian Gulf and Oman Sea in the south (18). The province has a total of area 70,697 km^2 and a population of 1,578,183 reported in 2011 (18). This area (27.1884°N 56.2768°E) has 13 counties and 14 islands in the Persian Gulf. Bandar Abbas is the capital city of the province and is located 0 to 20 meters above the sea level (18). Hormozgan Province experience a very hot and humid climate with temperatures sometimes exceeding 49 °C (120 °F) in summers (18). There is very little precipitation annually.

Sample collection

In this cross sectional study a total of 50 water samples (approximately 500 mL) were collected from tap water resources (25 samples from tanks and 25 samples from wells) of Hormozgan. All cities of the province including Minab, Haji Abad, Bandare Pol, Gheshm, Jask, Jagin, Bandar Abbas, Roudan, Lavan, Parsian, and Bandare Gang, and some rural regions were included. All samples were transferred to Protozoology laboratory of school of medicine at Shahid Beheshti University of Medical Science, Tehran, Iran, within 2 days and were stored at room temperature.

Filtration and cultivation

Immediately after transformation to the laboratory, approximately 250 mL of each water sample was filtered using a 1.6 µm pore size cellulose nitrate membrane. After filtration, the middle part of the membrane was cut out by sterile scissors and was cultured in the plate containing 1.5% non-nutrient agar (NNA) covered with a monolayer of heat killed *Escherichia coli* (as a food source for the amoeba) (12). In this study, Bacto-agar (Difco) and distilled water were used to prepare the medium. To incubate the samples, the plates were put at room temperature for up to 2 months.

Cloning and Microscopic examination

One week after incubation, the microscopic investigation was started and the daily investigation continued up to 2 months. Detection of *Acanthamoeba* spp. was performed according to page keys by using light microscope at 100× magnification (19). To achieve bacterial and fungal contamination free plates, and easy detection of the parasite, cloning was carried out by transferring a few amoeba to a fresh medium.

Results

Overall, out of 50 water samples, 14 (28%) samples were found to be positive for *Acanthamoeba* spp. by microscopic investigation. Two stages were characterized by their flat-shaped trophozoites with obvious acanthopodia and double layer cysts wall with star-shaped endocyst and round ectocyst, respectively. Out of 25 examined well samples 9 samples (36%) were found contaminated with *Acanthamoeba* spp., while only 5 (20%) of 25 tank samples were found positive. Contaminated samples were collected from following cities: Minab (2), Haji Abad, Bandare Pol, Gheshm, Jagin (3), Bandar Abbas (2), Roodan, Parsian, Lavan and Bandare Gang (table 1). Some water supplies were located in one county but provided drinking water for another county. However, contaminated water supplies were mainly drinking waters supply of rural and urban regions of Gheshm, Minab, Jask (two contaminated samples that were collected from Jagin were drinking water supply of Jask), Bandar Abbas and Bandare Gang counties. It is worth noting that in comparison with other contaminated supplies the water supply tank of Gheshm was highly contaminated.

Discussion

In our study, well samples were found more contaminated than tank sample. Less contamination of tank samples can be caused by disinfectant and usual washing of tanks. The present study revealed that water resources of all studied counties of the province were contaminated with potentially pathogenic *Acanthamoeba* species. Previously, potentially pathogenic *Acanthamoeba* species were isolated from different kinds of water resources in Iran (20-22). Contamination rate of water resources in some studies was in accordance with the present study. In the northwest of Iran, Behniafar et al. reported 40% and 24% contamination of different water resources (both of recreational and tap waters) with FLAs and *Acanthamoeba* spp., respectively (23). Nazar et al. found 32% of parks and public squires water samples contaminated to *Acanthamoeba* (24). Another research in Shiraz city showed that 35 samples out of 120 samples (29%) were polluted with *Acanthamoeba* by using culture and Giemsa staining methods (25).

Also, Niyyati et al. in 2015 reported 38% contamination of different kind of Kish Island's water resources with *Acanthamoeba* (T11, T5, T4 and T3 genotypes) and *Vermamoeba vermiformis* in southern Iran (22). Relatively higher occurrence in comparison with the present study can be caused by the simultaneous investigation of two genera of FLAs.

As we see in the present study a relatively high percentage of water resources are contaminated with *Acanthamoeba* species in Hormozgan Province. Thus, water resources can be potential hazard and source of infection. Also, less contamination of tanks in comparison with wells represent relative efficacy of normal disinfectant. However, contamination of disinfected waters reflects

resistance of *Acanthamoeba* against normal disinfectant. More researches should be conducted in Hormozgan province to determine genotypes of *Acanthamoeba* species using molecular methods.

Conflict of interests

Authors declare no conflict of interests.

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References

1. 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):1-26.
2. Marciano-Cabral F, Cabral G. Acanthamoeba spp. as agents of disease in humans. Clin Microbiol Rev 2003;16(2):273-307.
3. Nuprasert W, Putaporntip C, Pariyakanok L, Jongwutiwes S. Identification of a novel T17 genotype of Acanthamoeba from environmental isolates and T10 genotype causing keratitis in Thailand. J Clin Microbiol 2010;48(12):4636-40.
4. Marciano-Cabral F, Jamerson M, Kaneshiro ES. Free-living amoebae, Legionella and Mycobacterium in tap water supplied by a municipal drinking water utility in the USA. J Water Health 2010;8(1):71-82.
5. De Jonckheere JF. Molecular identification of free-living amoebae of the Vahlkampfiidae and Acanthamoebidae isolated in Arizona (USA). Eur J Protistol 2007;43(1):9-15.
6. 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(4):1109-17.
7. Alizadeh H, Apte S, El-Agha M-SH, Li L, Hurt M, Howard K, et al. Tear IgA and serum IgG antibodies against Acanthamoeba in patients with Acanthamoeba keratitis. Cornea 2001;20(6):622-7.
8. Chappell CL, Wright JA, Coletta M, Newsome AL. Standardized Method of MeasuringAcanthamoeba Antibodies in Sera from Healthy Human Subjects. Clin Diagn Lab Immunol 2001;8(4):724-30.
9. Brindley N, Matin A, Khan NA. Acanthamoeba castellanii: high antibody prevalence in racially and ethnically diverse populations. Exp Parasitol 2009;121(3):254-6.
10. Stockman LJ, Wright CJ, Visvesvara GS, Fields BS, Beach MJ. Prevalence of Acanthamoeba spp. and other free-living amoebae in household water, Ohio, USA—1990–1992. Parasitol Res 2011;108(3):621-7.
11. Pelletier JS, Miller D, Liang B, Capriotti JA. In vitro efficacy of a povidone–iodine 0.4% and dexamethasone 0.1% suspension against ocular pathogens. J Cataract Refract Surg 2011;37(4):763-6.
12. Khan NA. Acanthamoeba: biology and increasing importance in human health. FEMS Microbiol Rev 2006;30(4):564-95.
13. Khan NA. Acanthamoeba: biology and pathogenesis. 1 st ed. London: Caister Academic Press; 2009.
14. Rezaeian M, Niyyati M. Pathogenic Free Living Amebas In Human. Tehran: TUMS Publication; 2010.
15. Winiecka-Krusnell J, Linder E. Bacterial infections of free-living amoebae. Res Microbiol 2001;152(7):613-9.
16. Horn M, Wagner M. Bacterial Endosymbionts of Free-living Amoebae. J Eukaryot Microbiol 2004;51(5):509-14.
17. Rezeaian M, Farnia S, Niyyati M, Rahimi F. Amoebic keratitis in Iran (1997-2007). Iran J Parasitol 2007;2(3):1-6.
18. Wikipedia. https://www.wikipedia.org/ 2017.
19. Page FC. A new key to freshwater and soil gymnamoebae: with instructions for culture. 1988.
20. Badirzadeh A, Niyyati 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(2):1.
21. Solgi R, Niyyati M, Haghighi A, Taghipour N, Tabaei SJS, Eftekhar M, et al. Thermotolerant Acanthamoeba spp. isolated from therapeutic hot springs in Northwestern Iran. J Water Health 2012;10(4):650-6.
22. Niyyati 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 2015:8(s1):1-6.
23. Behniafar H, Niyyati M, Lasjerdi Z, Dodangeh S. High occurrence of potentially pathogenic free living amoebae in water bodies of kaleybar and khodaafarin, east azerbaijan province. Curr World Environ 2015;10(Special Issue 1 (2015)):727-31.
24. 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(3):603-8.
25. GHADAR GJS, Solhjoo K, NOROUZ NM, Rohi R, ZIA JS. Isolation and identification of free living amoeba (Naegleria and Acanthamoeba) in Shiraz water resources by morphological criteria. PARS J Med Sci 2012;10(3):33-41.

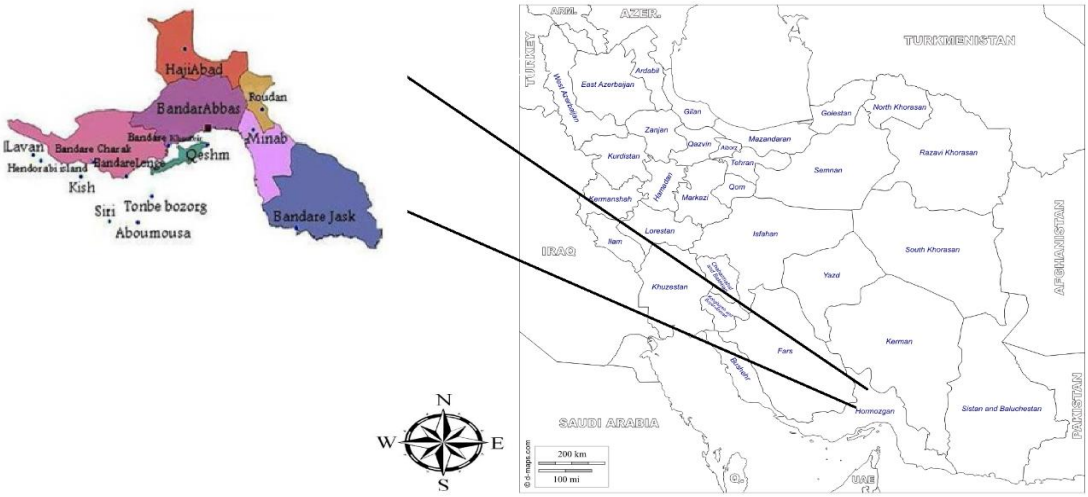

Figure1. Position of Hormozgan Province in Iran

Table1. Details of contaminated water supplies to Acanthamoeba in Hormozgan province

Kind of water supply	Number of samples (Number of contaminated samples)	Locations of contaminated samples
Tank	25 (5)	Bandare Pol Jagin Gheshm Roudan Parsian
Well	25 (9)	Minab (2) Jagin (2) Lavan Haji Abad Bandar Abbas (2) Bandare Gang