

Original Article

Isolation of *Acanthamoeba* Spp. from Different Environmental Sources

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Abstract

Background: *Acanthamoeba* spp. are free-living amoebas found in a wide variety of natural habitats. The high percentage of *Acanthamoeba* in different environmental sources represents a sanitary risk for public health especially contact lens users and immunocompromised patients. The aim of this study was to determine the presence of *Acanthamoeba* spp. in different environments such as water, soil, dust and ophthalmology wards.

Methods: From March to November 2007, 80 samples were collected from numerous localities in Tehran city including university campus, Laleh park and ophthalmology center. Sample types were water, soil, dust, cow faeces and medical instrument. Each sample was filtered through nitrate membrane and cultured on 1% non-nutrient agar. These plates were followed up daily for 2 weeks. Monitoring continued for two months on a weekly basis.

Results: Overall, 46.25% of samples contained *Acanthamoeba* spp. All of the soil samples had shown positive culture in contrast to tap water. Of 61 dust samples, 28 were positive. Interestingly, we were able to isolate *Acanthamoeba* in treatment unit of an ophthalmology center in Tehran. It should be mentioned that two cow faeces showed positive culture as well.

Conclusion: The widespread distribution of *Acanthamoeba* spp. across the environmental sources and increasing numbers of HIV⁺ patients and contact lens wearers, as well as its ability as a pathogen carrier for humans, demands more awareness and knowledge for public as a risk for human health.

Keywords: *Acanthamoeba*, Environment, Risk factor, Iran

Introduction

A *canthamoeba* spp. are free living amoeba that are found in a wide variety of natural habitats including water, soil, dust, vegetables, hospital units, ventilation areas and sewage (1-4). The cyst form of this opportunistic amoeba can persist for years in the environment, since it has the ability to survive in harsh surroundings (3, 4). This clearly shows that human being often encounters *Acanthamoeba*. It should be mentioned that these amoebas have been isolated from nasal passages, skin and upper airways of healthy people as well (5).

However, *Acanthamoeba* is the causative agent of keratitis, pneumonitis and fatal granulomat-

ous encephalitis (5, 6). Human diseases caused by *Acanthamoeba* continue to rise worldwide, which is probably due to increasing populations of contact lens wearers and HIV⁺ patients (2). A ten-year study by Rezaeian et al. (2007) in Iran revealed that there was a considerable increase in the incidence of amoebic keratitis during the recent years (7). Of interest, there are many cases of amoebic keratitis, which occurs after swimming in contaminated pools, usage of homemade saline for washing contact lenses and exposure to dirt and dust (1). Therefore, the ubiquity of these organisms can be hazardous for human.

Besides, *Acanthamoeba* serves as a carrier for different pathogens such as *Legionella*, *Pseudomonas* and *Helicobacter* (8, 9). These bac-

terial pathogens can lead to severe human disease or manifest as complications of amoebic keratitis. Indeed, these amoebas can transfer different microorganisms to humans and to date, *Acanthamoeba* is introduced as a vehicle for circulation of pathogens between human and environment (10). The aim of this study was to determine the presence of *Acanthamoeba* spp. in different environmental sources such as water, soil, dusts, ophthalmology hospital units etc. This finding lead to additional researches for investigating presence of pathogenic *Acanthamoeba* strains in environment sources by molecular methods which can be a risk factor for people especially contact lens wearers and immunocompromised patients.

Materials and Methods

Sampling

From March to November 2007, 80 samples were collected from numerous localities including an eye center, university campus (Tehran University) and Laleh Park in Tehran City. The samples were from tap water, swimming pool, soil, dust, cow faeces, and medical instruments. These samples were examined in the in the laboratory of Protozoology Unit, Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran.

Processing of Samples

Water samples consist of approximately 500 ml volume were filtered through a cellulose nitrate membrane. A piece of membrane was placed on the agar medium directly. The samples collected by swab were vortexed in distilled sterile water in separate tubes. The suspensions were then filtered according to above procedure. other

samples including soil and dust were also dissolved in distilled sterile water and filtered for further examinations.

Culture

Non-nutrient agar (NNA) was prepared with Amoeba Page Saline. Amoeba Page Saline consist of 2.5 mM NaCl, 1 mM KH₂PO₄, 0.5 mM Na₂Hpo₄, 40 µm CaCl₂-6H₂O and 20 µm MgSO₂.7H₂O. The final pH of this solution was adjusted to about 6.9 with KOH.

The filters were placed on 1% medium and the plates were incubated at room temperature. Then, the plates were monitored for detection of trophozoites or cysts of amoeba daily until 14 days and followed up weekly for 2 months.

Results

The identification of *Acanthamoeba* at the genus level in this study was based on distinctive features of double walled cysts. The cysts were detected usually after one week, although in some of the samples, cysts were identified after one month. Probably this is due to different genotypes of these amoebas.

From 80 samples that were studied in different environmental locations in Tehran, 37(46.25%) contained *Acanthamoeba* (Table 1). All soil samples (n=5) had positive cultures in contrast to tap waters which were all negative (Table 2). It should be mentioned that 2 positive water samples were from swimming pools. Of 61 dust samples, 28 were positive. Interestingly, *Acanthamoeba* was isolated from different wards of an eye center in Tehran including treatment units (Table 3). It should be mentioned that 2 cow faeces collected in this study have showed positive culture.

Table 1: Frequency of samples according to their localities and number of positive samples

Locality	No.	Positive (%)
Ophthalmology center	30	8 (26.6)
Other localities*	50	29 (58)
Total	80	37(46.25)

* Other localities except ophthalmology center

Table 2: Sample types and the frequency of positive samples

Samples	No.	Positive	%
Soil	5	5	100
Water	6	2*	33.3
Dust	61	28	45.9
Cow faeces	2	2	100
Others	6	0	0.0
Total	80	37	46.25

* Pool waters were positive

Table 3: Frequency of different samples from the eye center in Tehran

Eye center samples	No	Positive
Tap Water	2	0
Soil	2	2
Dust *	20	6
Others	6	0
Total	30	8

* Dusts from treatment unit and flowers were positive

Discussion

In this study, we have recovered *Acanthamoeba* spp. from a variety of ecological habitats using culture methods.

Acanthamoeba has been isolated from numerous ecological sources such as tap water, coastal water, swimming pools, ponds, soil, dust, dirt, food, lens solutions, hospital units and even chlorinated water (1-4, 11). The high percentage of *Acanthamoeba* in different environmental sources represents a sanitary risk for public health especially contact lens users and immunocompromised patients (10). Of interest, obtained results of this study showed that dust and soil sources have a high percentage of *Acanthamoeba* spp. This can explain that there is a sanitary risk for people who meet nature (10), since there are reports concerning occurrence amoebic keratitis after exposure to contaminated dirt and dust (12). In the present study, we have isolated *Acanthamoeba* from dust samples of treatment unit in

an ophthalmology center in Iran. It is worth mentioning that corneal surgeries combined with exposure to *Acanthamoeba* are the two major risk factors for developing amoebic keratitis (1, 2). In addition, the flower dusts have showed positive culture. Public health education is advisable in order to avoid bringing flowers for patient's visit in eye surgery and recovery units to prevent eye contact with flower dusts. Besides, proper disinfections such as H₂O₂ 3% in ophthalmology hospital units should be implicated since chlorinated disinfections are not able for killing *Acanthamoeba*.

The isolation of *Acanthamoeba* from cow faeces in this study highlights a possible transmission of *Acanthamoeba* through animal faeces. This result is in agreement with another research where pathogenic and virulent strains of *Acanthamoeba* were isolated from the rectum of wild animals such as squirrel (10). This finding suggests human's close contact with animals as an amoeba-spreading vehicle may be hazardous.

Maghsood et al. examined 12 water samples in Iran by molecular methods and revealed that T2 genotype was the most frequent genotype in their samples (13).

Overall, the widespread distribution of *Acanthamoeba* spp. across the environment and increasing numbers of HIV⁺ patients and contact lens wearers as well as its ability to act as a carrier for human pathogens reminds us of the need for increasing the public's awareness and knowledge and to educate them about *Acanthamoeba*'s risk to human health. This study can also serve as a platform for further investigation and research, such as determination of the genotypes of the isolated *Acanthamoeba*.

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