

Isolation and morphotyping of *Acanthamoeba* spp. and *Vermamoeba* spp. from hospital air-conditioning systems

Hastane klima sistemlerinden *Acanthamoeba* ve *Vermamoeba* türlerinin izolasyonu ve morfotiplendirilmesi

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SUMMARY

Objective: The aim of the study was to investigate the presence of free-living amoebae (FLA) such as *Acanthamoeba* and *Vermamoeba* in air conditioning systems of Cumhuriyet University Research and Application Hospital (CURAH).

Method: For this purpose, twenty-four dust samples were collected using sterile cotton swaps from grilles and filters of five different unit air-conditioners of CURAH located in Sivas, Turkey. Samples were transferred in a sterile tubes to the laboratory and spread onto non-nutrient agar (NNA) plates with a lawn of inactive *Escherichia coli*. Culture plates were incubated at 30°C. FLA genera was identified according to morphotyping keys.

Results: FLA were found in 14 (58.3%) of examined 24 air-conditioner. Ten (41.6%) were identified as *Vermamoeba* spp, 4 (16.7%) as *Acanthamoeba* spp., after microscopic examination.

Conclusions: These findings should alert us on the risk of *Acanthamoeba* and *Vermamoeba* infections. In addition, these amoebas could be host for bacteria, fungi and viruses and indirectly become agents of transmission for these pathogens. For decrease the risk both *Acanthamoeba* and their endosymbionts, regularly cleaning of air-conditioner filters and grilles using effective disinfectants are essential.

Keywords: *Acanthamoeba* spp., *Vermamoeba* spp., Air-conditioning system

ÖZET

Amaç: Çalışmada, Cumhuriyet Üniversitesi Araştırma ve Uygulama Hastanesi klimalarında *Acanthamoeba* ve *Vermamoeba* gibi serbest yaşayan amiplerin (SYA) varlığının araştırılması amaçlanmıştır.

Yöntem: Bu amaçla, hastanenin beş farklı ünitesinden toplam 24 klimanın ızgara ve filtre kısımlarından steril eküvyonlarla sürüntü örnekleri alınmıştır. Alınan sürüntü örnekleri steril tüplerde laboratuara getirilmiş ve üzerine inaktive edilmiş *E.coli* içeren Besleyici Değeri olmayan Agar plaklarına yayılmıştır. Ekim yapılan plaklar 30°C de inkübe edilmiştir. Üreme olan plaklardan pasajlar yapılmıştır. Saptanan amipler morfotiplendirme anahtarları kullanılarak cins düzeyinde tanımlanmıştır.

Bulgular: İncelenen 24 klima örneğinin 14'ünde (%58.3) SYA saptanmıştır. Bunlardan 10'unda (%41.6) *Vermamoeba* spp, 4'ünde (%16.7) *Acanthamoeba* spp. tanımlanmıştır.

Sonuç: Bu bulgular klima sistemlerinde potansiyel patojen SYA ler açısından dikkatli olunması gerektiğini göstermektedir. Ayrıca, bu amipler içlerinde patojen bakteri, mantar ve virusları taşıyabilmektedir. Hem SYA hem de içlerinde taşıyabilecekleri endosimbiontlarıyla ilgili riskleri azaltmak için havalandırma sistemleri düzenli olarak etkili dezenfektanlarla temizlenmelidir.

Anahtar Sözcükler: *Acanthamoeba* spp., *Vermamoeba* spp, Klima sistemleri

INTRODUCTION

Free-living amoebae (FLA) such as *Acanthamoeba*, *Naegleria*, *Balamuthia* and *Sappinia* have been isolated from many different environment and clinical samples: soil, air, fresh water, tap water, ear, lung secretions, nasopharyngeal mucosa samples¹⁻⁴. They have been associated with infections of the central nervous system in humans⁵⁻⁷. *Acanthamoeba* and *Naegleria* are the most common FLA genuses². But *Balamuthia* and *Sappinia* are less common causes of clinically significant infections. *Naegleria fowleri* is the only species of *Naegleria* that causes the disease was called primary amoebic meningoencephalitis (PAM)⁶. *Acanthamoeba* infections involves the skin and central nervous system in immunosuppressed patients. They are causative agents of granulomatous amoebic encephalitis (GAE). Also occasionally involves the cornea in relatively healthy patients by causing *Acanthamoeba* Keratitis (AK). Another genus is *Vermamoeba*: *Vermamoeba vermiformis* is potential pathogen in humans⁸. Bacterial pathogens such as *Legionella pneumophila*, *Pseudomonas* spp., *Staphylococcus* spp, *Proteus* spp. and some *Mycobacteria* spp. are taken up by FLA without undergoing digestion. These bacteria multiply within FLA thus increasing the probability of causing infections in humans⁴.

The air-conditioner is essential in living indoor area for comfort and as a symbol of development and modern lifestyles. On the other hand, air conditioning systems has been shown to be the source of air-born bacterial, viral etc. diseases. Therefore, in the present study, we analysed dust samples obtained from air-conditioning systems of Cumhuriyet University Research and Application Hospital (CURAH).

METHODS

In the study, twenty-four dust samples were collected using sterile cotton swabs from grilles and filters of five different unit air-conditioners of CURAH located in Sivas, Turkey.

Collection of air-conditioner swab samples

Samples (in total N=24) were collected from air-conditioning systems between June-September 2011. Swab samples were obtained from the inner surface, grills and filters of air-conditioners using sterile cotton swabs. Samples were taken from an area of approximately 50 cm². Then these swabs were placed in the sterile plastic tubes containing 2mL of sterile buffer solution. The samples were stay overnight at room temperature in the

parasitology laboratory. Four dust samples were obtained from air conditioners of Blood centre, 3 dust samples were from Haematology laboratory, 8 dust samples were from Biochemistry laboratory, 9 dust samples were from Radiology units in September 2011.

The incubation of samples and culture

For the culture of samples non-nutrient agar (NNA) was used. NNA was prepared with page ameba saline (PAS) (2.5mM NaCl, 1mM KH₂PO₄, 0.5mM Na₂HPO₄, 40μM CaCl₂·6H₂O and 20μM MgSO₂·7H₂O). Agar was dissolved in PAS (1.5%), autoclaved and dispensed onto sterile plates. The dust samples were stayed overnight at room temperature. The day after sampling day, the tubes were santrifuged at 1000 rpm for ten minutes. To isolate FLA, pellet were placed on NNA plates containing a layer of inactivated *Escherichia coli* as a food source. The plates were incubated at 30°C.

Isolation and passages

After two days of incubation, the plates were examined for the detection of trophozoites or cysts of FLA. Plates were examined daily. Once growth was spotted approximately 1 cm² of agar containing the FLA trophozoites or/and cysts was taken from the grown culture and placed at new NNA plates with a lawn of heat-killed *E.coli*. Detected FLA were maintained by serial passages in this medium.

Identification

For identification of FLA, the movement and structural properties of amoebas were analysed. The cyst and trophozoite morphology of FLA were examined using light microscope and the genera was identified according to morphotyping keys⁹⁻¹².

Statistic analysis

The data obtained from this study using SPSS (Ver: 22) I installed the program. Khan-square test was used to evaluate the data. Groups Fisher's exact test was used for differences between and p <0.05 was considered significant.

RESULTS

FLA were recovered from 14 (58.3%) out of 24 swab samples. *Acanthamoeba* spp. were identified in 4 (16.7%), and *Vermamoeba* spp. in 10 (41.6%) with morphotyping (Figure 1,2). The units of taken the dust samples and the distribution of obtained FLA were given Table 1. There was no difference between the groups performed the statistical analysis.

Acanthamoeba cyst forms were typical with a star-like shape and very easy to differentiate as Group I and II. But *Vermamoeba* cyst forms was more similar on NNA.

Identification of *Acanthamoeba* trophozoites at the genus level is relatively easy due to the presence of

spiny surface projections, called acanthopodia, on trophozoites. But, using only morphological criteria, identification of these amoebae at the species level has been difficult. The genus of *Acanthamoeba* have been placed into three morphological groups (I,II and III) based on cyst size and shape⁹⁻¹².

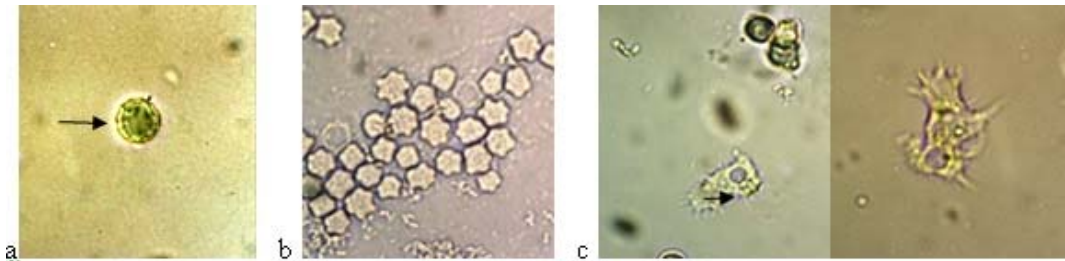


Figure 1. *Acanthamoeba* spp. cyts obtained from samples, a. Cyst in saline(40x),b. Cyts on NNA (40x).c. *Acanthamoeba* trophozoites in saline(40x)

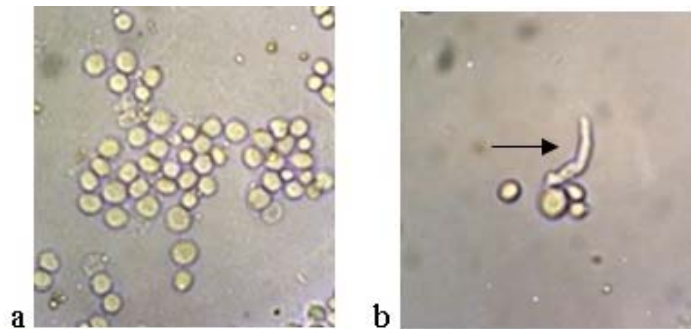


Figure 2. *Vermamoeba* spp. a.Cysts on NNA, b.Trophozoites in saline (40x)

Table 1. The source of air-conditioner samples and identified FLA

Source	FLA		<i>Acanthamoeba</i>				<i>Vermamoeba</i>	
	(+)	(-)	No		No		No	
	No	%	No	%	No	%	No	%
Unit 1	3	12.5	1	4.17	1	4.17a	2	8.33a
Unit 2	2	8.3	1	4.17	1	4.17a	1	4.17a
Unit 3	4	16.7	4	16.7	1	4.17a	3	12.5a
Unit 4	5	20.8	4	16.7	1	4.17a	4	16.7a
Total	14	58.3	10	41.6	4	16.67a	10	41.66a

a: No differences were found between groups, Ffisher’s exact test, 2-sided

The genus of *Vermamoeba* trophozoite is monopodial; body subcylindrical with circular cross-sections; no lateral wrinkles, never has adhesive uroid⁹. They had a hyaline anterior region almost always longer than broad. Cyst forms of

these amoebae genus have smooth-walled, which were spherical or ovoid. The median of cyst diameter was 8-10µm. Cyst walls were mostly homogeneous¹⁰.

DISCUSSION

Free-living amoebae, ubiquitous and widely distributed protozoa, feed on bacteria, algae, fungi, and small organic particles and are adaptable to their environment. FLA have been isolated from samples obtained from environmental materials such as pools, lakes, thermal springs, domestic water supplies, soil, air, dust, air-conditioning units¹⁻⁷. To our knowledge the presence of FLA in the environment does not mean a risk factor for illness. However, many species of *Acanthamoeba* are potentially pathogenic for animals and for humans. The prevalence of FLA have been reported to be 30 % from environmental waters and tap water systems in Sivas^{13,14}. Also in the other study from Sivas isolated *Acanthamoeba* spp. obtained from tap water systems as pathogen T4 type have been reported^{13,14}. The prevalence of FLA have been reported to be between 23% and 89% from water systems in the world¹. *Legionella* bacteria present within FLA can survive and become resistant to inappropriate environmental conditions. Moreover, FLA play a major role in *L. pneumophila* transmission. By Burak and Zeybek, the presence of *L. pneumophila* and FLA have been examined in water and swab samples obtained from shower heads in 61 houses in İstanbul. *L. pneumophila* have been isolated from 21.3% of houses examined and FLA from 31%. But no significant correlation have been found between the presence of *L. pneumophila* and FLA¹⁵.

In Iran 13 strains have been isolated from dust samples and classified at the genotype level. Results revealed the presence of T4, T5 and T11 genotypes within these samples¹⁶. During the harmattan in Nigeria, the presence of FLA was reported obtained from air and isolated a total of 38 strains of FLA by Lawande¹⁷, 21 of the genus *Acanthamoeba*, eight *Naegleria*, four *Schizopyrenus*, three *Didascalus* and two *Tetramitus*. Three pathogenic species, *A. culbertsoni*, *N. fowleri* and *A. rhyodes*, were also reported¹⁷. El Sibae reported presence of *Acanthamoeba polyphagia* in the cooling towers of the air conditioning systems¹⁸. According to these researchers FLA may be source for numerous outbreaks of air-born diseases such as the aspergillosis, Legionnaires' disease and Severe Acute Respiratory Syndrom caused by SARS virus.

In Malaysia, Chan et al. have been isolated *A. castellani*, *A. culbertsoni*, *A. griffini*, *A. hatchetti* and *A. polyphaga* from air-conditioners. They have detected *Acanthamoeba* spp in 20 out of 87 dust

samples. All *Acanthamoeba* spp. samples have been found pathogen T4 type¹⁹.

Astorga et al. have also been isolated FLA from air-conditioning units from Chile. They have collected water and air samples from air-conditioning systems and checked for the presence of *Acanthamoeba* spp. Positive samples have been further classified at the genotype level after sequencing the highly variable diagnostic fragment 3 (DF3) region of the 18S rRNA gene²⁰.

CONCLUSIONS

In this study the presence of FLA in air condition system of CURAH was investigated. Twenty-four dust samples were collected using sterile cotton swaps from grilles and filters of five different unit air-conditioners of CURAH located in Sivas, Turkey. FLA were found in 14 (58.3%) of examined 24 air-conditioner. Ten (41.6%) were identified as *Vermamoeba* spp, 4 (16.7%) as *Acanthamoeba* spp., after culture and microscopic examination. This is the first time *Acanthamoeba* and *Vermamoeba* spp. have been isolated from air-conditioning systems in Turkey.

The public health importance of FLA is explained in two ways. First, FLA are themselves opportunistic pathogens in humans. Second, FLA serve as reservoirs for pathogenic bacteria and virus. So, the filters of air-conditioner must be clean frequently. Overall, the widespread distribution of potentially pathogenic FLA in the studied source demands more awareness within the health professionals in Turkey as this pathogen is emerging as a risk for human health worldwide.

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