

Short Communication

Isolation of Vahlkampfiids (*Willaertia magna*) and *Thecamoeba* from Soil Samples in Iran

M Niyati¹, J Lorenzo-Morales², A Motevalli Haghi¹, B Valladares², *M Rezaeian¹

¹ Dept. of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

² University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Tenerife, Canary Islands, Spain

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Abstract

Background: Vahlkampfiids contains wide variety of genres with some known as human pathogens such as *Naegleria*, *Vahlkampfia* and non pathogens such as *Willaertia*. Since there was no evidence of presence of Vahlkampfiids in different sources in Iran, we have analyzed soil samples to clarify the presence of these amebas.

Methods: Seven soil samples collected in Tehran were analyzed to clarify the presence of Vahlkampfiids in soil sources, using microscopic examination of non nutrient agar cultures and specific Vahlkampfiids primer pair.

Results: Vahlkampfiids were detected in 2 out of 7 soil samples by direct examination of cultures. Sequence analysis confirmed that *Willaertia magna* (*W. magna*) was present in 2 samples. Additionally, *Thecamoeba* were detected in all of soil samples.

Conclusion: To the best of our knowledge, this is the first report of existing *W. magna* and *Thecamoeba* in Iran. Over all, more research should be implicated in Iran for identification of Vahlkampfiids within different environmental sources as well as their pathogenic capability relevant for human beings.

Keywords: *Vahlkampfiids*, *Thecamoeba*, Iran

Introduction

Free-living amoebae have been classified into different families with a wide variety of morphological and physiological characteristics (1). *Amoeba* families with known medical significance include Vahlkampfiidae and Acanthamoebidae (1, 2). *Acanthamoeba* are ubiquitous amebas classified into 15 different genotypes known as T1-T15 (2). To date, it has been proven that the T4 genotype is the most important causative agent of

sight threatening amoebic keratitis (AK) and Granulomatose Amoebic Encephalitis (GAE) (2).

Previous studies on this genus revealed that potentially pathogenic *Acanthamoeba* has a widespread distribution in environmental sources such as dust, water and soil in Iran and worldwide (3-5). *Thecamoebidae* is the other family of free living amoebae without any medical importance (1). However, it should be noted that these amebas can be a hazard for other free living amebas such as

*Corresponding Author:

Email: rezaeian@sina.tums.ac.ir, Telephone: 009821-88951392

Acanthamoeba and *Balamuthia* and act as prey for *Thecamoeba* (Unpublished data). Vahlkampfiids also have a wide variety of genuses with some known as human pathogens such as *Naegleria* and *Vahlkampfia* (2). *Willaertia magna* (*W. magna*) and *Tetramitus* has been isolated from environmental sources as well (1). It should be mentioned that except *Naegleria*, there are only a few morphologically distinctive features within different genuses of Vahlkampfiids making proper identification by microscopic examination a difficult task (6). Genus identification based on cyst morphology requires a lot of experience (1, 6). Therefore, molecular based methods are promising for identification of different genus in Vahlkampfiids (6). Since there was no evidence of presence of Vahlkampfiids in different sources in Iran, we have analyzed soil samples using specific primer pair of Vahlkampfiidae families in order to clarify the presence of these amoebas.

Material and Methods

Samples

Seven soil samples were collected from different locations in Tehran during our previous study (3). Briefly, soil samples were dissolved in distilled sterile water and filtered as described previously (3).

Microscopic examination

Microscopic examination of the Non Nutrient Agar plates using inverted microscope revealed that *Acanthamoeba* cysts were present after three weeks. Besides, in two plates numerous spherical cysts different to the usual shape of *Acanthamoeba* cysts were observed. The different morphology of the cysts in the latter plates was indicative of presence of a different cyst other than *Acanthamoeba* cyst, most likely Vahlkampfiids.

Acanthamoeba strains were already genotyped in our previous study using DF3 primer pair (4, 5). The spherical cysts from two plates were cloned by transferring them to many other replicate. It is worthy to mention that amongst the *Acanthamoeba* and the suspected Vahlkampfiids numerous amoeba from the Thecamoebidae family were also present, phagocytosing the *Acanthamoeba* and Vahlkampfiidae family.

DNA Extraction, Polymerase Chain Reaction and Sequencing

The round cysts suspected to be Vahlkampfiidae were harvested by pH 7 PBS using a cell scraper. Centrifugation in 3000 rpm for 15 minutes was performed. The supernatants were then discarded and 500 µl DNA lysis buffer (50 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0) and 30 µl proteinase K (0/25 mg/ml) were added to the precipitants and incubation were done at 60°C, overnight. DNA extraction was performed by phenol-chloroform method as previously described (4, 5). PCR reaction was performed using Vahlkampfiids specific Primers (7). The nucleotide sequence of primers was as follows:

Vahl-F: 5'GTCTTCGTAGGTGAACCTGC3', Vahl-R: 5'CCGCTTACTGATATGCTTAA3'.

PCR reaction was set up in a 30 volume containing 1.25 U Taq DNA polymerase, 30 ng DNA, 4mM MgCl₂, 200 M dNTP and the cycles were set up as following: an initial denaturing step at 95 °C for 2 min and 30 repetitions at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s with an elongation step of 5 min at 72 °C in the last cycle. Electrophoresis was performed using 2% agarose gel. PCR products were then purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using a MEGABACE 1000 automatic sequencer (Healthcare Biosciences, Barcelona, Spain) in the University of La Laguna Sequencing Services (Servicio de Secuenciación SEGAI,

University of La Laguna). Genus and species identification was based on the homology analysis of amplified fragment in comparison to the available DNA sequences in GenBank.

Results

Microscopic examination of 2 out of 7 plates revealed Vahlkampfiids cysts (Fig. 1). *Thecamoeba* trophozoites were also detected in all soil samples (Fig. 2). Vahlkampfiid cysts

showed a round ectocyst and endocyst and the size were up to 20 μm long. *Thecamoeba* were ovoid flat in shape with an obvious nucleus in the anterior end of this amoeba. Sequence analysis of PCR-products (Fig. 3) using Basic Local Alignment Search Tool (BLAST) confirmed that the spherical cysts genomic sequence is highly homologous with *W. magna* gene available in the gene data bank (Accession number: [X96579](#)) thus confirming the microscopic observation.

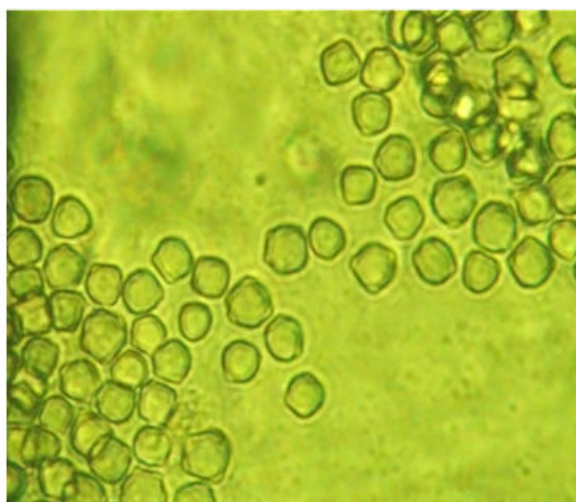


Fig. 1: Vahlkampfiid cysts in soil sample (Non Nutrient Agar) X 400

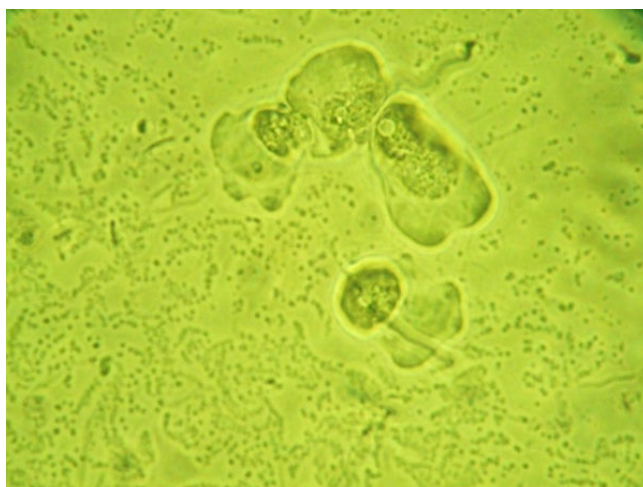


Fig. 2: *Thecamoeba* in soil sample (Non Nutrient Agar) X 400

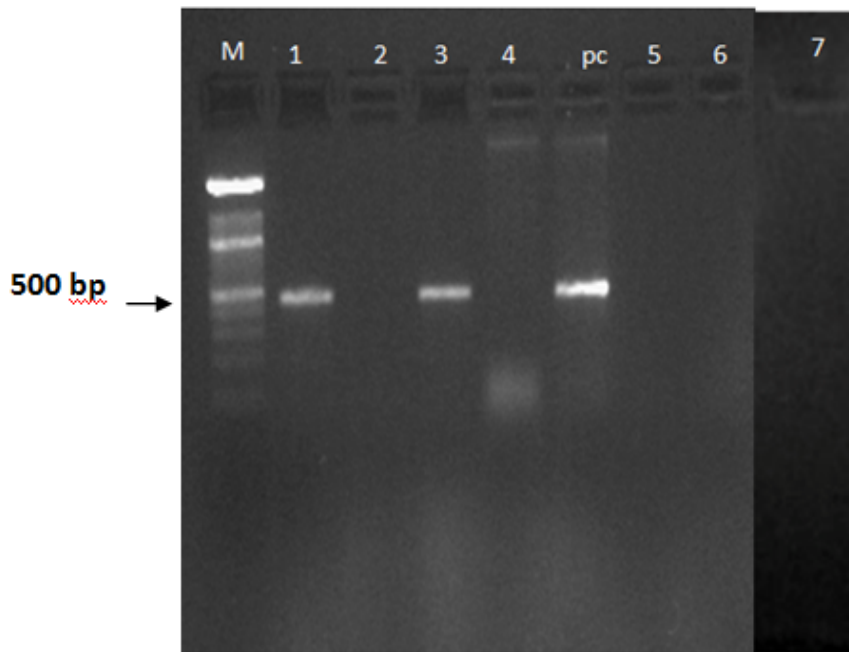


Fig. 3: PCR-product of soil samples with specific Vahlkampfiids primer pair
M=molecular Weight Marker
PC=Positive control
Numbers= code of soil samples

Discussion

It is already well known that *W.magna* has a widespread distribution in different environmental sources (6). However, to the best of our knowledge, this is the first report of existing *W. magna* and *Thecamoeba* in Iran. There are also reports regarding isolation of *W. magna* from bovine feces and thermally polluted waters (2). Although there is discrepancy in different researches regarding pathogenic ability of this genus, there are reports speculating pathogenicity of *W. magna* in an immunocompromised dog isolated from stomach ulcers (2). It is suggested that corticosteroid administration resulting in immunodeficiency provided the opportunity for *W. magna* in ulcer development. However, no reports of pathogenic ability of *W.*

magna in human and experimental mice have been reported yet (2).

W. magna is known as a thermophilic amoeba morphologically very similar to *Naegleria gruberi* (1). One of the main differences between *W. magna* and *Naegleria* is their flagellate stage. *W. magna* develops four flagella during the flagellate stage in contrast to *Naegleria* developing two flagella (1). Cysts of this amoeba have a thick inner wall layer and a loose spherical or polygonal outer wall layer. The size of the amoeba form can reach up to 100 μm and the cysts up to 18-28 μm long (1). It should be noted that *Thecamoeba* were detected in all soil samples. No cyst form of this amoeba has been detected yet (1). A big size (up to 170 μm) and different nuclear pattern are

amongst the unique morphological characteristics of *Thecamoebidae*.

In conclusion, more research should be implicated in Iran for identification of Vahlkampfiids within different environmental sources as well their pathogenic capability relevant for human beings.

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