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## First Record of Keratitis Caused by *Valkampfia* in Iraq

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## RESEARCH ARTICLE

# First Record of Keratitis Caused by *Valkampfia* in Iraq

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**ABSTRACT**

*Acanthamoeba* is the most well-known causes amoebic keratitis. However, other free-living amoebas (FLA), such as *Valkampfia*, can also causes of keratitis. Twenty-two samples were collected from cases suspected of amoebic keratitis in Kirkuk Hospital to investigate the presence of *Acanthamoeba*. Cultivation process was carried out directly on non-nutrient agar (NNA) medium supported with killed *Escherichia coli* bacteria. Inverted and light microscope were used at 40X magnification to diagnose cultures growth. Only two samples showed positive growth on NNA medium. However, no any presence of *Acanthamoeba* was observed as were expected using polymerase chain reaction (PCR) which amplified by JDP, the specific *Acanthamoeba*'s primers. These two samples were further investigated using primers of internal transcribed spacer (ITS) addition to sequence technique, which confirmed the presence of *Valkampfia* spp. Remarkably, this discovery was registered in the American Gene Bank (NCBI) as inaugural infection that occurrence in Iraq and reported as the first case despite its global rarity. The findings of this study highlight the importance of considering to diagnosis the other species of free-living amoebae caused amoebic keratitis and treatment. Also, further research is needed to better understand the epidemic of keratitis caused by *Valkampfia*.

**Keywords:** *Acanthamoeba*, Cornea, Free living amoebae, Keratitis, *Valkampfia***Introduction**

*Valkampfia* is a free-living amoeba, a single-celled organism that lives in soil and water. It belongs to the same family as *Naegleria*.<sup>1,2</sup> *Valkampfia* has been isolated from soil and water in Iran and Japan.<sup>3,4</sup> Although most common cause of amoebic keratitis (AK) associated with *Acanthamoeba* with improper usage of soft contact lenses such as taking shower or swimming in suspected water sources while wearing lenses.<sup>5,6</sup> *Valkampfia* can infect the eye causes keratitis which leads to visual disturbances and blindness.

The risk of *Valkampfia* keratitis increases with contact lens wear, swimming in contaminated water, and using non-sterile contact lens cleaning solutions. Infection can also occur after accidental corneal scratches.<sup>7</sup> Symptoms of *Valkampfia* keratitis include painful corneal sores, redness, excessive tearing, foreign body sensation, photophobia, and blurred vision.<sup>8</sup> Doctors typically diagnose *Valkampfia* keratitis

by examining a corneal scraping under a microscope and/or performing molecular diagnostics, to prevent *Valkampfia* keratitis, people should keep their contact lenses in a sterile solution and avoid wearing them while swimming or in saunas.<sup>2</sup> Treatment for *Valkampfia* keratitis depends on the severity of the infection. In superficial infections, doctors may remove infected and damaged corneal cells. In mild to moderate cases, medical treatment alone may be sufficient to eradicate the infection. However, severe cases may require surgery.<sup>9</sup> This study aimed to determine the species of free-living amoeba (FLA) that caused keratitis in patients and its prevalence.

**Materials and methods***Samples collection and cultivation*

Twenty-two corneal samples were collected from patients suspected of amoebic keratitis in Kirkuk

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Hospital from July 2021 until January 2022. A small portion of the infected corneal tissue was scraped by special ophthalmic forceps under the supervision of specialized medical staff and cultured directly on non-nutrient agar media which were prepared with weight NaCl (2.5mM) 120 mg, MgSO<sub>4</sub>. 7H<sub>2</sub>O (20 μM) 4 mg, CaCl<sub>2</sub>. 2H<sub>2</sub>O (40 μM) 4 mg, Na<sub>2</sub>HPO<sub>4</sub> (0.5 mM) 142 mg, KH<sub>2</sub>PO<sub>4</sub> (1 mM) 136 mg) the salts were dissolved in 1000 ml of D.W. and 15 g of DIFCO agar, (pH = 6.9). Then, they were autoclaved at 121°C and supplemented with killed *Escherichia coli* bacteria which were prepared depending on.<sup>5</sup> The samples were incubated at 30°C for one to two weeks.

### Macroscopic examination

Macroscopic examination of growth cultures on non-nutrient agar (NNA) was performed using an inverted and light microscope at 40x magnifications to distinguish positive FLA growth from negative growth. Negative growth cultures were excluded, while the positive growth cultures were seen in a trophozoite stage and that been smaller than the *Acanthamoeba*'s trophozoite were kept for molecular diagnosis.

### Molecular diagnosis

#### DNA extraction

For DNA extraction one milliliter (mL) of suspended free-living amoebae in phosphate buffer was added to 1.5 mL Eppendorf tubes and centrifuged at 2000 g for 5 minutes to remove the supernatant. Then, the DNA extraction is completed according to the protocol of the DNA extraction kit (Promega- USA).<sup>3</sup>

#### Primers and polymerase chain reaction protocol

In the first step, JDP primers known were used to identify *Acanthamoeba* isolates using conventional polymerase chain reaction.<sup>2</sup> But after the negative results which were showed that its free of *Acanthamoeba*, then the second step were carried out by primers called ITS (Internal Transcribed Spacer) as an experimental method instead of JDP primers. ITS primers are used to amplify the internal transcribed spacer region of ribosomal RNA (rRNA) genes, which can be used to identify and characterize a wide range of microorganisms.<sup>8</sup> The Primers (Macrogen, Korea) sequences were used to amplify as follows Table 1:

**Table 1.** Sequence of primers.

Primers	Sequences
JDP -F	(5'-GGCCAGATCGTTTACCGTGAA-3')
JDP -R	(5'-TCTCACAAAGCTGCTAGGGAGTCA-3')
ITS -F	(5'-TCCGTAGGTGAACCTGCGG-3')
ITS -R	(5'-GCTGCGTTCCTCATCGATGC -3')

program of conventional PCR was performed using the following Table 2:

**Table 2.** PCR program

Steps	°C	Time (min:sec)	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:40(JDP)–00:30(ITS)	30
Annealing	56 (JDP)– 55 (ITS)	00:45(JDP)–00:30(ITS)	
Extension	72	00:55(JDP)–00:30(ITS)	
Final extension	72	07:00	1

polymerase chain reaction products were compared with the base pairs of DNA ladder marker ranged between 1500 bp - 100 bp, which has been previewed using UV instrument after electrophoresis process adding 1% agarose gels under 100 Volt/ Milliampere at 75 min and stained with ethidium bromide.

### Sequencing

polymerase chain reaction products were sequenced by Sanger method using ABI3730XL automated DNA sequencer by Macrogen Corporation – Korea. The results were analyzed using Geneious version of the software.

## Results and discussion

### Visual examination

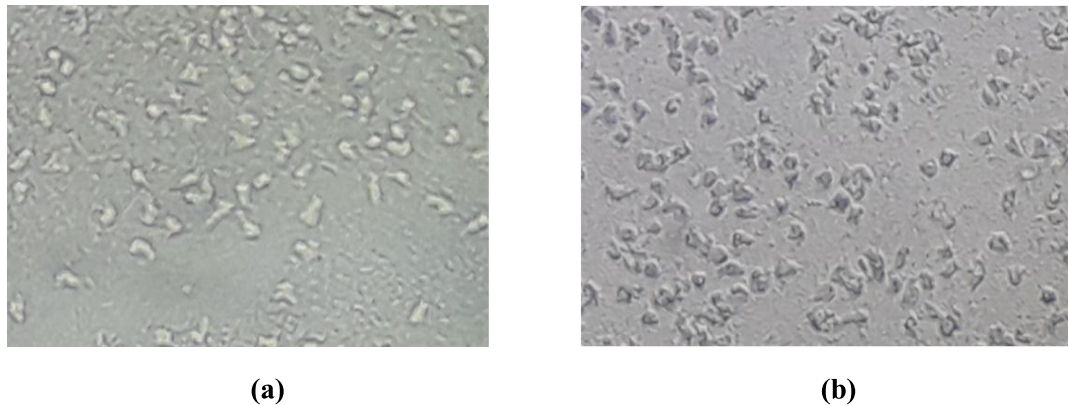
Patients suspected of amoebic keratitis were diagnosed based on their clinical symptoms, which included redness, burning, persistent pain, tears, and blurred vision with form a white layer covered partially above and around the cornea Fig. 1.



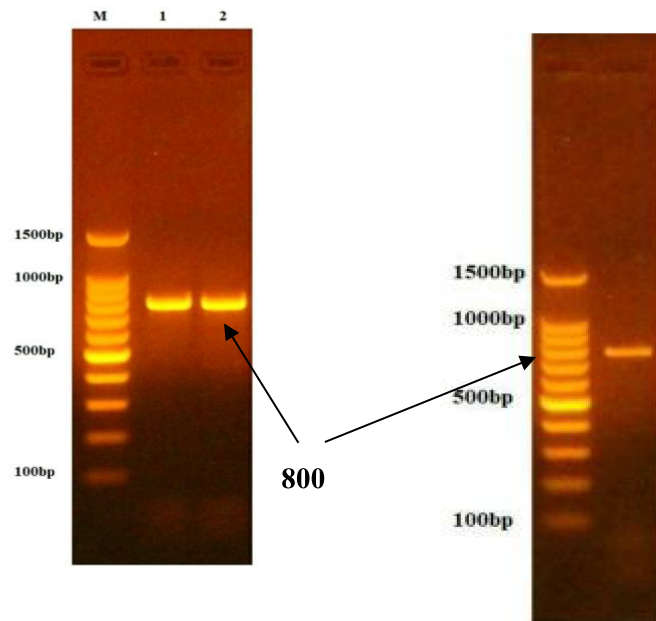
**Fig. 1.** Amoebic keratitis shows redness and a white layer above and around the cornea.

### Macroscopic examination

Two samples of free-living amoeba were isolated from patients by culturing on non-nutrient agar medium and examined using inverted and light microscope at 40x magnification and the other twenty



**Fig. 2.** The trophozoites of free living amoeba (*Valkampfia* spp.), which appear growth on non-nutrient agar media under: (a) Inverted microscope & (b) Light microscope at 40X.



**Fig. 3.** Electrophoresis of positive samples after using ITS primers and conventional PCR amplification. (M):Marker, 1 & 2: The two positive samples at 800bp.

remaining of negative samples for amoeba growth were discarded [Fig. 2](#).

#### *Molecular examination*

polymerase chain reaction amplification using ITS primers followed by electrophoresis for two identical isolates that had previously examined positive for growth on NNA agar medium [Fig. 3](#).

#### *The genotyping of the isolated samples*

The positive samples were sequenced using the Sanger method. The sequences were edited using Geneious version of the software and compared to

sequences in the GenBank database. The isolates were identified as *Valkampfia* sp. and registered in GenBank under the accession numbers OK054349 and OL826792.

#### *Data analysis*

BLAST and FASTA results of *Valkampfia* sp. which were reported as a first case of keratitis and recorded at the location of National Center for Biotechnology Information [Figs. 4 to 6](#).

*Acanthamoeba* is the most common free-living amoebae that causes keratitis in humans.<sup>10,11</sup> However, other free-living amoebae, such as *Valkampfia*, can also cause this infection.<sup>12,13</sup> This study

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Vahlkampfia sp. isolate Iraq internal transcrib...	Vahlkam...	1304	1304	100%	0.0	100.00%	706	OK054349.1
Vahlkampfia sp. AK-2007 genes for ITS1_5.8S...	Vahlkam...	1212	1212	99%	0.0	98.00%	714	AB330067.1
Vahlkampfia sp. isolate TY Iraq internal transc...	Vahlkam...	1173	1173	100%	0.0	96.61%	709	QL826792.1
Vahlkampfia sp. AK-2007 genes for ITS1_5.8S...	Vahlkam...	996	996	99%	0.0	92.47%	712	AB330066.1
Vahlkampfia sp. isolate VS10 internal transcri...	Vahlkam...	985	985	96%	0.0	92.74%	686	QL415191.1
Vahlkampfia sp. Va5 gene for ITS1_5.8S rRNA...	Vahlkam...	981	981	99%	0.0	92.01%	742	LC191900.1
Vahlkampfia sp. Va3 gene for ITS1_5.8S rRNA...	Vahlkam...	981	981	98%	0.0	92.10%	739	LC191898.1
Vahlkampfia sp. isolate NWSS ITS1.ab1 inter...	Vahlkam...	972	972	97%	0.0	92.14%	768	MF187528.1
Naegleria sp. isolate A1.4a 18S ribosomal RN...	Naegleri...	972	972	99%	0.0	91.78%	727	KT356270.1

Fig. 4. BLAST result of Valkampfia sp. which have accession numbers (OK054349) and (OL826792).

**Vahlkampfia sp. isolate Iraq internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

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CGGTCGTCGCTCCCTCCTCCGGGAGGGGTGGAGACGCTCCGACACCCGATAGCGAACGGTCCAAGTCCCTTCCC
GGGACCTCGAGGACTCCGGTCTCGAACCATCCCGGGGAGACTCCAAAAAACCGTAGCCCCCTCGGCGAAACCAA
CATGGATGGAGGGATCGCTCGTCGATCCTTTCAGAAACGAGAATAACCTAGTCGACTACTTTCGACGACGGATACCT
CGGTTCCCACGCCGATGAAGAGCGGCGGCGAAACGCGATATGTCGTGCGAATCGCAGAATTCGGCGAATCACGCAATC
CTCGAACGCATCCGGCGCTCTCGGGTCTTCCCGGGAGCATGCCCGTCTGAGCGTCGTTCCCAAAAAATCCCAACCTC
GATGGTTCGAAACCATCCGGGGAAGGGTGATCGGGAGTCTGCAGAAATGCAGTCTTCTGGGAACGAGGGCCACCCT
CTCGAGCCCCGTCGACGACGAGGGACCAGGTGCTTAGCTTCCGTCGAGAGCGCCTCAGGGTGCAACTCACATCGG
GAGTCCATGCCCTGATCCATTTCAACTCTCTTCGACCTCGGGATCAGCTCCGGAGTGGACAGACGCCGTCGGTGGTC
CGTGAGACCAGGGTCGGAGGAGTCGCATCTCCCTCTCTAACCCAGACCTCAGATCGGGCAAGATTACCCGCTGAA
CTTAAGCATATCATAA
    
```

Fig. 5. FASTA sequence the isolate of Valkampfia sp. number 1 (OK054349).

**Vahlkampfia sp. isolate TY Iraq internal transcribed spacer region, partial sequence**

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CGGTCGTCGCTCCCTCCTCCGGGAGGGGTGGAGACGCTCCGACACCCGATCGCGAACGGTCCAAGTCCCTTCCC
GGGACCTCGAGGACTCCGGTCTCGAACCATCCCGGGGAGACTCCAAAAAACCGTAGCCCCCTCGGCGAAACCAA
ATGGATGAAGGGATCGCTCGTCGATCCTTTCAGAAACGAGAATAACCTACTCGACTACTTCTACGACGGATACCTAC
GGTTCACGCCGATGAAGAGCGAGGCGAAACGCGATATGTCGTGCGAATCGCAGAATTCGGCGAATCACGCACTCC
TCGAACGAATCCATCCCGCTCTCGGGTCTTCCCGGGGCGATGCCCGTCTGAGCGTCGTTCCCAAAAAATCCCAACCTC
CGATGGTTTCGAACCATCCGGGGAAGGGTGATCGGGAGTCTGCAGAAATGCAGTCTTCTGGGAACGAGGGCCACCCT
TCTCGAGCCCCGACCGACCATGAGGGACCAGGGGCTTAGGTGCCGTCGAGAGCGCCTCAGGGTGCAACTCACATCG
GGAGTCCATGCCCTGATGCATTTCAACTCTCTTCGACCTCGGGATCAGCTCCGGAGTGGACAGACGCCGTCGGTGGT
CCGTGATACCAGGGTCGGAGGAGTCGCATCTCCCTCTCTAACCCACTACCTCAGATCGGGCAAGATTACCCGATGA
ACTTAAGCATATCATAA
    
```

Fig. 6. FASTA sequence the isolate of Valkampfia sp. number 2 (OL826792).

describes the two first cases of Valkampfia keratitis in Iraq. The two cases of Valkampfia keratitis were from different locations in Kirkuk, indicating that the infection may be spread in more than one city in Iraq. The first patient was a forty-year-old farmer from a village near of Kirkuk, and the other patient was a university student from the city of Kirkuk. Neither patient had a history of swimming, but the university student had worn contact lenses two years ago. It is unclear whether contact lens wear was a contributing factor in this patient's infection because there was a space of time between the date of wearing lens and the date of infection. The farmer's daily contact with soil and irri-

gation water when working in agriculture is the most likely explanation for his infection. *Valkampfia* is a rare free-living amoebae that can cause keratitis, a serious infection of the cornea. There is limited research on Valkampfia keratitis, especially clinical cases. A review of the NCBI database revealed that all registered samples of *Valkampfia spp.* were environmental isolates from soil or water.<sup>14</sup> However, there are studies that have proven the pathogenicity of the organism and its caused of amoebic keratitis. In Egypt, environmental models of *Valkampfia* were used on experimental laboratory animals to determine the effect of *Valkampfia* which were caused keratitis in

animals.<sup>15</sup> In addition, co-infection of *Acanthamoeba* and *Valkampfia* were isolated from two cases in a study at an institution in Spain.<sup>16</sup> Another case of a mixed infection of *Acanthamoeba* and *Valkampfia* were isolated from a cosmetic contact lens wearer in Iran.<sup>17</sup> In this study, *Valkampfia* was registered in the American Gene Bank as the first reported case despite the global rarity of this infection. Also, it was free of mixed infection with *Acanthamoeba*.

The findings of this case report highlight the importance of considering *Valkampfia* as an important cause of keratitis, especially in patients who are contact lens wearers or who have a history of swimming in non-tropical countries. Further research is needed to develop diagnosis, better understanding the epidemic and strategies treatment of *Valkampfia* keratitis.

## Conclusion

Keratitis is a common inflammation of the cornea that can be caused by a variety of pathogens, including bacteria, viruses, and fungi. However, in some cases, keratitis may be caused by free-living amoebae, which are a diverse group of protozoa that can be found in soil, water, and other environmental sources. In Iraq, keratitis caused by free-living amoebae may be underdiagnosed, as clinicians may not be aware of this possibility or may not have access to the necessary diagnostic tests. Therefore, it is important to investigate all cases of keratitis microscopically and to use molecular diagnostic methods to detect the causative organism. It is also important to note that *Valkampfia* is not the only rare species of free-living amoeba that can cause keratitis. Other rare species such as *Hartmannella* can also be responsible. Therefore, clinicians should be aware of the possibility of infection with these rare species, especially in patients with keratitis who do not respond to standard treatment regimens.

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## Authors' declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Fig-

ures and images, that are not mine, have been included with the necessary permission for republication, which is attached to the manuscript.

- Author(s) sign on ethical consideration's approval.
- No animal studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Kirkuk Water Directorate.

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## اول تسجيل لألتهاب القرنية الناجم عن فالكامفيا في العراق

تركان قاسم قرياغدي

قسم السيطرة النوعية الميكروبيولوجية لمياه محافظة كركوك ، العراق .

### المخلص

الايكانثاميبيا هي المسبب الأكثر شيوعاً لالتهاب القرنية الأميبي. ومع ذلك ، فإن الأميبات الحرة المعيشة (FLA) الأخرى ، مثل فالكامفيا ، يمكن أن تسبب أيضاً التهاب القرنية. تم جمع 22 عينة من مرضى يشتبه بإصابتهم بالتهاب القرنية الأميبي في مستشفى كركوك للتحقق من وجود الايكانثاميبيا المسببة للالتهاب . وقد تمت زراعة العينات مباشرة على وسط أجار غير مغذي (NNA) مدعم ببكتيريا الإشريكية القولونية الميتة. لغرض تشخيص نمو الأوساط الزرعية تم استخدام المجهر المقلوب والمجهر الضوئي بقوة تكبير 40X. أظهرت عينتان فقط نمواً إيجابياً على وسط NNA ، الا انه لم يظهر أي وجود للايكانثاميبيا كما كان متوقفاً باستخدام تفاعل البلمرة المتسلسل (PCR) الذي تم تضخيمها بواسطة (JDP) ، وهي بادئات متخصصة في تحديد الايكانثاميبيا. تمت دراسة هاتين العينتين بشكل أوسع باستخدام بادئات فاصل النسخ الداخلي (ITS) مع تقنية التسلسل (sequencing)، والتي أكدت وجود *Valkampfia sp*. ومن اللافت للنظر أن هذا الاكتشاف تم تسجيله في بنك الجينات الأمريكي (NCBI) باعتباره أول إصابة تحدث في العراق وتم الإبلاغ عنها كأول حالة على الرغم من ندرة هذه العدوى عالمياً. نتائج هذه الدراسة تسلط الضوء على أهمية النظر في تشخيص الأنواع الأخرى من الأميبات الحرة المعيشة التي تسبب التهاب القرنية وعلاجها. ايضاً هناك حاجة إلى مزيد من البحث لفهم وباء التهاب القرنية الناجم عن فالكامفيا بشكل أفضل.

**الكلمات المفتاحية:** الايكانثاميبيا، قرنية، اميبا الحرة المعيشة، التهاب القرنية، فالكامفيا.