

FIRST CASES OF *ACANTHAMOEBA* KERATITIS IN SLOVAKIA

František Ondriska¹, Martin Mrva², Martin Lichvár³, Peter Žiak⁴, Zuzana Murgašová⁵,
Eva Nohýnková⁶

¹HPL (Ltd) Microbiological Laboratory, Bratislava, Slovak Republic

²Department of Zoology, Comenius University, Bratislava, Slovak Republic

³Hospital and Polyclinic, Bratislava, Slovak Republic

⁴Faculty Hospital and Jessenius Faculty of Medicine of Comenius University, Martin, Slovak Republic

⁵Faculty Hospital, Nitra, Slovak Republic

⁶Department of Tropical Medicine, Charles University, Prague, Czech Republic

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Abstract: We present the case report of the first identification of *Acanthamoeba* as a causative agent of keratitis in the Slovak Republic. For the first time, *Acanthamoeba* sp. Group III was isolated from a 53-year-old patient with keratitis, which was manifested after an injury of the right eye. A delayed visit to a physician as well as a late diagnosis of the illness led to the advanced stage of eye disease. As the treatment with itraconazol and cornea transplantation showed no result, enucleation of the eye was decided. *Acanthamoeba ludgunensis* was also the causative agent of keratitis in a 39-year-old patient wearing contact lenses. His complaints occurred a month after bathing in a thermal swimming pool. The symptoms presented in the left eye were those of herpetic keratitis, and led to a cloudy cornea with circular infiltrate and poor vision. A prompt clinical and laboratory diagnosis, along with treatment with propamidine-isetionate resulted in a significant improvement of the eye condition. Contact lenses were probably related to another case of *Acanthamoeba* keratitis. The patient, a 15-year-old girl, kept wearing contact lenses during bathing in various swimming pools and in the sea; her contact lenses were also regularly washed under tap water. Due to the fact that cysts of *Acanthamoeba* sp. group II were found in the contact lens solution, this is presumed to be the source of the eye infection.

Address for correspondence: František Ondriska, RNDr, PhD, HPL (Ltd) Microbiological Laboratory, Istrijská 20, 841 07 Bratislava, Slovak Republic.
E-mail: ondriska@hpl.sk

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INTRODUCTION

Free-living ubiquitous amoeba of the genus *Acanthamoeba* are under medical attention, not only as causative agents of chronic infection of the central nervous system - granulomatous amoebic encephalitis (GAE), but also as causative agents of serious, eye devastating disease. While the GAE occurs more often in children with mental

disorders or in immunocompromised patients, in healthy people, amoebic keratitis is mostly the result of amoebic cornea colonisation.

Species of the genus *Acanthamoeba* are free-living protozoa spread world-wide. Their life cycle has 2 forms: trophozoite and cyst. They occur practically everywhere: in soil, dust, all kinds of water - drinking water, lakes, rivers, thermal and brackish waters or in the sea. We can

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find them in heating, air conditioning and moisturising systems, even in dialysing and contact lens media. It has been proved that under certain conditions *Acanthamoeba* species could become a reservoir of virulent bacteria of the genus *Legionella*, even in domestic water sources [20]. Under unfavourable conditions, the trophozoites revert into inactive cysts, and thanks to them the *Acanthamoeba* is able to withstand extreme environment conditions (-20 to 42°C). Both mentioned forms occur in the epithelium of the invaded cornea [19, 21, 22, 37]. The eye could be infected by direct contact with amoeba through an injured cornea, by contaminated water, air or contact lenses. The incubation period is unknown.

Acanthamoeba keratitis is a chronic infection of cornea. Man may be infected regardless of gender, age or race. The long development and unilateral presentation are typical for the disease occurring as a result of the direct invasion of amoebae into the eye tissue, and penetration to the corneal epithelium. In many cases, a small lesion of the cornea could present a point of entry for the amoeba, which could, for example, be caused by previous infection of herpes simplex, bacteria, fungi, trauma of eye, or small bruises caused by contact lenses. Amoebae could enter the eye during swimming in contaminated water or by incorrectly kept and disinfected contact lenses. Without an early diagnosis and specific therapy, a painful keratitis develops and on the cornea there appears an arched or circular infiltrate with ulceration. After the ulcer perforation, a serious eye inflammation could result in damage to the eye and a threat to the eyesight [1, 2, 11, 13, 21, 33].

Since *Acanthamoeba* keratitis was first diagnosed in 1973 [17], the number of cases has grown steadily. A dramatic increase is recorded after the year 1980 in relation to increased frequency of the use of contact lenses [35, 42], and also due to improved diagnostic methods. Contaminated lenses are usually the first step in the pathogenesis of *Acanthamoeba* keratitis. In contact lenses users, *Acanthamoeba* keratitis occurs 20 times more often compared to people who do not use contact lenses. For example, in Great Britain the occurrence of *Acanthamoeba* keratitis in people who do not wear contact lenses is around 1.20 in a million of adult people, and around 20 in a million of those who wear contact lenses [29]. In the USA, the occurrence in contact lenses users is significantly lower (1.36 in a million). The real occurrence of *Acanthamoeba* keratitis is probably higher [10].

The most significant factors for *Acanthamoeba* keratitis - corneal trauma and contact lenses are related with the first isolations of amoeba of the genus *Acanthamoeba* as causative agents of human keratitis in the Slovak Republic, of which the case histories are presented.

CASE DESCRIPTION

Case 1. In April 1999, a 53-year-old man thoroughly rinsed his right eye in a disused unkept well after trauma

by a wooden splinter and did not pay it any further attention. Due to increasing pain and worsening visual acuity, in August 1999, he visited an ophthalmologist who locally treated the keratitis. But after progression of the disease, he was admitted to hospital in October 1999. Visual acuity of the right eye became so poor that the patient was able to count fingers only at the distance of 2 metres. A semilunar lesion was found on the corneal epithelium and several whiteish lesions on the endothelium. In the anterior eye chamber, a mild inflammation was present, pupil reaction was slower, fundus reflex was darkened, and retinal details were not visible due to changes in the anterior chamber. Intraocular pressure was normal. The patient suffered severe pain radiating to the forehead. The left eye stayed intact. Apart from the medical finding of eye, there was an itching rash on the skin of the whole body, which was diagnosed and histologically verified as pemphigus herpetiformis. The patient noticed the occurrence of the skin disease shortly before the eye incident. Treatment by antibiotics was started: amoxicillin with clavulan acid (375 mg daily for 5 days) later, due to unchanging medical findings, ciprofloxacin was used (500 mg daily for 10 days). Gentamycin (eye drops) was applied locally for 3 days, then ofloxacin (eye drops) for 12 days. Dexametasonum was also applied (500 mg per day for 10 days) and atropinium sulfuricum 1% drops for the whole duration of hospitalization. The skin was treated by loratadine tablets (once daily) and triamcinoloni acetamidum cream locally.

During hospitalization, haematological, biochemical, microbiological and immunoserological tests were carried out on the patient. Generally, no significantly increased CRP (under 5000 mg/l), positive rheumatoid factor by latex screening (RF turbidimetrically 48.500 IU/ml) were recorded. All other tests were negative. Objectively, no positive progress of the eye status was recorded during hospitalization. The patient was complaining of intermittent eye pain and worsening of visual acuity to counting fingers right before the eye. The intraocular pressure of the affected eye moved in the area of higher values, and due to this acetazolamidum tablets were applied. Strong, mixed injection of the eye persisted, the cornea was strongly edematous, the precipitates of endothelium were unchanged, and in the anterior chamber there was a thick inflammatory reaction. The status was defined as idiopathic necrotising sclerokeratolysis of the right eye. Within approximately 3 days, the cornea ulcer perforated along with a small iris protrusion. The visual acuity worsened regarding hand movement closely to the eye. After reepithelising of the cornea ulcer and taking a picture of the leucoma on right eye (Fig. 1), a symptomatic treatment - atropinium sulfuricum drops and hypromelozum, plus acidum boricum plus natrium tetraboricum drops was started.

Because this treatment did not improve the clinical finding, we focused our attention on possible *Acanthamoeba* etiology of the keratitis. In December 1999, we inoculated the cornea scratch on plates with NN

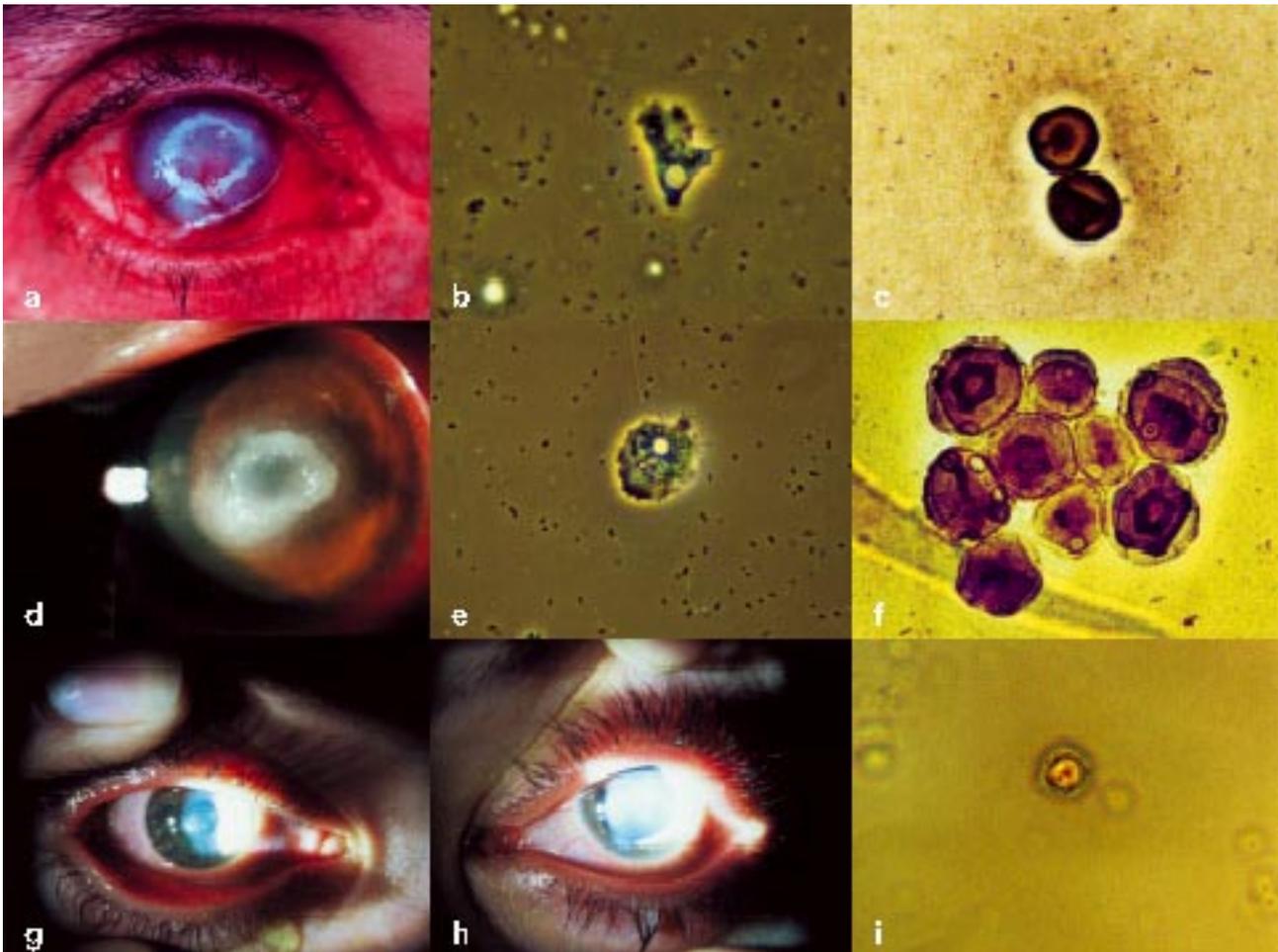


Figure 1. a. Case 1. Appearance of ring corneal ulcer in a right eye; b. Case 1. Trophozoite of *Acanthamoeba* sp. group III; c. Case 1. Protargol-stained *Acanthamoeba* sp. group III cysts; d. Case 2. Circular corneal infiltrate in a left eye; e. Case 2. Trophozoite of *Acanthamoeba lugdunensis*; f. Case 2. Protargol-stained *Acanthamoeba lugdunensis* cysts; g. Case 3. Corneal infiltrate in pattern of stripe in the right eye; h. Case 3. Corneal infiltrate in pattern of stripe in the left eye; i. Case 3. Cyst of *Acanthamoeba* sp. group II.

agar with a suspension of heat-inactivated *E. coli*. After 48 hrs incubation at 37°C, we microscopically observed the trophozoites and, after 10 days cultivation the amoeba cysts as well, which, according to morphological characteristics we identified as *Acanthamoeba* sp. group III. (Fig. 2-3). A biopsy sample from the cutaneous lesion was microscopically and culturally negative. Due to the advanced stage of the disease, itraconazol was applied (200 mg daily in 2 doses for 5 weeks), as well as loratadinum tablets, ibuprofenum 400 mg tablets as needed. Locally we applied polymyxin B plus neomycine plus phenylephrin, hypromellose drops 5 times daily into the conjunctive lobe of the right eye. Subjectively, the patient felt a partial improvement, confirmed by checks repeatedly carried out in January 2000 by cultivation samples from cornea on NN - agar presence of *Acanthamoeba* sp. group III. Because of these results, we continued the therapy with the mentioned preparation. Control examination after the therapy in April 2000 did not prove the presence of amoeba in the samples. According to the well-developed stage of the disease, the treatment brought very slow improvement – subjective

regression of pain and temporary improvement of vision. After keratoplasty carried out without any result, we decided to enucleate the globe.

Case 2. In May 2001, a 39-year-old man was treated at an eye clinic diagnosed with keratitis profunda in the left eye of unclear etiology, similar a herpetic infection. The patient used to wear contact lenses (type for 14 days wear) for several years, which he regularly tended. In anamnesis, he quoted bathing in a thermal pool with lenses approximately one month before the first symptoms. Subjectively, he felt severe stabbing pains in the eye, photophobia and vision deterioration. Symptoms were: significant eye redness, cornea containing circular infiltrate measuring 4 mm, and deterioration of vision (Fig. 4). The illness did not respond to topical therapy with aciclovirum (cream) and corticoids. The right eye did not show any signs of pathogenous changes. Cultivation of the eye swab revealed a polyresistant strain of *Pseudomonas aeruginosa* and *Pseudomonas* sp. Local therapy was applied to the patient by ofloxacin (eye drops) every 10 minutes, in combination with cephalosporin

(eye drops) and intravenously ciprofloxacin (100 mg every 12 hrs). The local therapy was complemented by enema of the conjunctiva pouch with 2% povidone iodine (3 times daily) and by 1% homatropine. Due to no improvement in the clinical finding, we focused on potential infection by *Acanthamoeba*. Cultivation of a corneal scrape on NN agar with *Escherichia coli* revealed massive findings of amoebae, identified as *Acanthamoeba lugdunensis* (Fig. 5-6). On the basis of this result, the treatment with itraconazole was started, this being the only available drug at the time. After acquiring propamidine isethionate (Brolene), this was applied in 0.1% concentration as eye drops every 30 minutes and as a cream at night, in combination with ofloxacin (eye drops) and cephazolin (eye drops). Itraconazole was discontinued because it created a whitish deposit on the edges of the cornea defect and signs of slowing epithelialisation. In the course of 3 weeks, an improvement was noted, severe ocular pain was regressed and vision improved – the patient saw the fingers at a distance of 2 m. Repeated cultivations of the corneal scrapes did not reveal any amoebae. The local therapy was utilized until August 2002 because signs of initiation of satellite infiltrations occurred when we tried to stop the treatment. The condition of the eye continued to improve, the cornea cleared (Fig. 7), and significant improvement of vision was noted (counting fingers at a distance of 0.5 m). The healing of the centrally localized defect changed the curvature of the cornea with consequent hypermetropic shift. The defect completely corrected the patient's myopia (-8.5).

Case 3. A 16-year-old female patient. Never had any eye problems. From the age of 7 she had worn a myopic correction for both eyes, and since February 2000 had used contact lenses. She always kept the lenses in freshly-prepared solution, but the contact lens case had been washed in tap water. She wore the lenses even during bathing in swimming-pools, lakes and the sea. In July 2001, the first complaints occurred: eye redness, sensitivity to light and lacrimation. There was no pain. After the local application of o-chloramphenicol cream, an improvement was noted. Despite this, the patient kept bathing with contact lenses, even in the sea water. In August 2001, complaints occurred repeatedly, such as eye redness, light sensitivity and lacrimation, which continued with variable intensity. She received therapy with fluorometholon drops, homatropin drops, hypromellose drops and AD vitamins 5 times a day. The right eye showed improvement, but not the left one. In the central cornea of the right eye a small zonule infiltration was found, and larger map infiltrate similar to pseudoherpetic keratitis in the left eye (Fig. 7-8). Visus of the right eye was 5/7.5, in left 5/15. In December 2001, corneal scrapings were taken from both eyes; cultivation on NN agar with *E. coli* showed presence of amoebae in the left eye; the right eye was intact. Cysts of amoebae were found in used contact lens solution. Amoebae had been

determined according to the cysts morphology as *Acanthamoeba* sp. group II. (Fig. 9). Encystations were observed on the 7th day; however encystations did not occur during passage on fresh NN - agar. Therapy with propamidine isethionate (Brolene drops, 5 times a day), in combination with neomycin and aciclovir was started. After 2 months, lacrimation and sensitivity to light stopped, eyes were without redness. The patient felt better, vision was also improved, in right eye to 5/5, in left eye to 5/10, and infiltrates became smaller.

MATERIAL AND METHODS

Corneal scrapes and contact lens solution were cultivated on 1.5% NN (non-nutrient) agar (Bacto Agar, Difco) with a suspension of heat-inactivated *Escherichia coli* [43]. Plates were examined microscopically for the presence of amoeba daily during 10 days.

Identification of amoebae was made on the basis of morphological criteria after Page [26] and Pussard & Pons [28] using protargol warm impregnation of cyst walls and PAT Agar for cyst pores visualisation.

Warm impregnation. Cysts suspension from older culture was mixed with glycerol - albumin on a slide and fixed for 2 hours by Clark fixation mixture [26]. After a short rinse in distilled water, the samples were impregnated in 0.5 % silver proteinate (protargol) for 2 hours at 60°C, then briefly (several seconds) immersed in developer (1% hydroquinone in 5% sodium sulphite), rinsed in distilled water, dehydrated and mounted on slides in damara or Canadian balsam.

PAT Agar. The fixation of cysts suspension in glycerol - albumin was carried out, made by the same method as for impregnation. Method of the next preparation was as follows: samples into 1% periodic acid for 10 minutes, rinsed for 15 min under tap water, 1 hour in 0.2% thiocarbohydrazide in 20% acetic acid, rinsed in 20%, 10% and 5% acetic acid, tap water (5 min) and in distilled water, 30 min in 0.5% silver proteinate (protargol) at room temperature, then developer (1% hydroquinone in 5% of sodium sulphite), rinsed in tap water, dehydrated and mounted on slides damara or Canadian balsam.

DISCUSSION

The dominant risk factor of *Acanthamoeba* keratitis is wearing contact lenses, and there is evident accumulation of the disease occurrence among young people. Infection of the cornea after injury, including surgical procedure [12, 38], occurs more frequently in adults, usually in advanced age [35]. According to Hansen and Kronborg [15], immunodeficiency could present a risk factor, even when an increased incidence of *Acanthamoeba* keratitis in patients with HIV was not recorded.

The disease of the first patient which, unlike, most cases documented so far, did not relate to wearing contact

lenses but developed after eye injury and its washing out with water from a disused well. However, we could reisolate the amoebae by cultivation of the water from this source, approximately one year after injury. Nevertheless, the splinter could also have been contaminated, the infection could have been the carrier of the amoebae, a carrier of the amoebae and have been affected and the eye injury could have helped their invasion into the cornea. There were mainly 2 factors responsible for the further development of the disease:

1. The patient went to a physician with an already advanced stage of the disease

2. The etiology of the disease was explained 10 months after the beginning of the infection, which significantly reduced specific therapeutic possibilities.

Similarly, as in most cases, the disease proceeded as herpes simplex keratitis and attention was directed at amoebae after using all other possibilities to search for the cause of the disease. The clinical picture was similar to most cases of keratitis caused by amoebae: atypical corneal abrasion with shaggy epithelium, eye status worsening, together with a feeling of a foreign body in the eye and severe pains, presence of characteristic anular infiltrate and corneal ulcer [3, 32]. A similar symptomatology was observed in the second patient, but with a difference - thanks to earlier determination of the etiologic agents and early therapy, the illness was successfully managed. The second patient, with the highest probability was infected through the contact lenses which he wore during swimming in thermal pools where the presence of *Acanthamoeba* was proved. It is not excluded that the progress of keratitis was helped by the infection of the bacteria *Pseudomonas aeruginosa*. It is known that in the etiology of keratitis a combined infection of *Acanthamoeba*, together with bacterial, viral or fungal pathogenous agents could occur [16, 40], while especially *Pseudomonas aeruginosa* in mixed infection with amoebae frequently causes keratitis [7, 11]. It is likely that in cases of mixed infections of *Acanthamoeba* and bacteria, the bacteria adhere to the surface of the cornea and thus significantly increase amoeba attachment to contact lenses, support their growth and could be the first step in pathogenesis of amoeba keratitis.

Thorough respecting of hygienic rules is a limiting factor of keratitis prevention in contact lenses users. The infection could be brought into the eye by improper treatment of contact lenses, e.g. by contaminated solution or cleansing by tap water. Water from tap-water or by home prepared physiological solutions are not suitable for cleaning contact lenses and could play an important role in lenses contaminated by *Acanthamoeba* and the origin of keratitis [9, 34, 39]. According to Radford *et al.* [29], disinfection, mainly using a proper disinfection solution for contact lenses, could prevent *Acanthamoeba* keratitis in 80% of cases. Niszl and Markus [24] and Beattie *et al.* [6] found not all solutions are suitable. Solutions must be reliably effective against both forms of the parasite and during the same time of exposure should reliably

eliminate *Acanthamoeba* cysts. Not respecting these rules probably caused the eye infection of the third patient, who not only wore contact lenses during bathing, but even did not take proper care of them. This was proved by the presence of amoeba cysts in the solution. The clinical finding in this patient was a little different. Unlike the previous 2 patients, both eyes were infected, even when amoebae were found only in one eye, and characteristic round infiltrate were absent.

Therapy of *Acanthamoeba* keratitis is a permanent problem. Its success depends on early identification because in the early stages of infection the trophozoites are more sensitive to therapy than the highly resistant protozoan cysts which accompany advanced infection [2]. One of the hypotheses of *Acanthamoeba* infection supposes [46] that the infection is not always caused by active amoeba infection, but also by persisting *Acanthamoeba* antigen. These cases could be even long term therapy ineffective. There are several therapeutic schemes, but with respect to the lack of our experience with the therapy of *Acanthamoeba* keratitis and due to the advanced stage in the first patient, we proceeded according to available literature sources [23, 40] and applied antifungal itraconazol in combination with locally applied antibiotics - neomycine and polymyxine B. In spite of negative cultivation after therapy, we recorded only a temporary eye status improvement. The improvement did not come after cornea transplantation [5, 33], therefore we decided to enucleate the eye. The pathological process in the eye caused by *Acanthamoeba* is at late therapy application (in this case approximately 10 months after infection) probably therapeutically hardly effective, and could be resolved only by radical - enucleation [41]. We were in a substantially different situation in the other 2 patients. Thanks to experiences with the first case, we diagnosed very quickly the causative agents and could therefore apply an early therapy. We used propamidine isethionate (Brolene) [44], even when other authors reported successful therapy after application of chlorhexidine [18, 31]. The application of propamidine isethionate in patients 2 and 3 turned out to be a suitable solution, mainly in the second patient where we recorded sight improvement. These cases confirmed that successful antiprotozoan therapy is limited by an early diagnosis and therapy.

There are several species of the genus *Acanthamoeba* capable of causing keratitis; however, a generally accepted and stable system for an exact strain determination is still a problem. There are several methods, from the diagnosis based on morphology [4, 26, 28], through immunodiagnostic methods, e.g. immunofluorescent colouring [14, 25, 30], up to the methods based on RNA and DNA analysis [8, 27, 36, 45]. We determined our isolates according to morphological criteria reported in the works of Pussard and Pons [28] and Page [26]. The determination was based on 2 ways of cysts membrane impregnation, which will help to differentiate its outer structure and pores. The above-mentioned works separate the particular species of

the genus *Acanthamoeba* into 3 groups (I, II, III) on the basis of characteristic morphology of outer and inner cyst membrane. In the case of the first patient, we identified the amoeba as *Acanthamoeba* sp. from group III. In the case of the second and third patients, there were different species of *Acanthamoeba* spp., which belonged to group II. The complete identification of the stem as *Acanthamoeba lugdunensis* [28] was successful only in the first patient. Unfortunately, there is no sufficiently reliable method of identification, and the variability of the cysts morphology could prevent this because it has not yet been included in the available determining manuals.

Acanthamoeba keratitis is a serious and painful eye disease regularly affecting healthy people. It is difficult to define any predisposition to the illness because of the ubiquity of the parasite, although wearing contact lenses is a clear factor increasing the risk of infection. Wearers should respect conditions of the disease prevention, especially the prevention of contact lenses contamination. On the other hand, determination of the causative agents with regard to the disease prognosis and success of therapy should be a quick, dynamic and complex process which depends on the level of education of the health personnel in this particular field.

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