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Case Report

The first *Acanthamoeba* keratitis case in the Midwest region of Brazil: diagnosis, genotyping of the parasite and disease outcome

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Abstract

We report an *Acanthamoeba* keratitis case associated with the use of contact lens in a 28-year-old female from Brasília, Brazil. Samples from corneal scraping and contact lens case were used for culture establishment, PCR amplification, and partial sequencing (fragments of ~400kb) of small subunit rDNA; both culture and PCR were positive. The sequence analyses of the cornea and of isolates from the contact lens case showed similarity with the T4 genotype. To the best of our knowledge, this is the first report of T4 *Acanthamoeba* keratitis case from the Midwest region of Brazil.

Keywords: *Acanthamoeba* keratitis. Culture. PCR.

INTRODUCTION

The daily use of contact lenses may favor the appearance of microtraumas in the cornea that could represent a gateway for the development of *Acanthamoeba* keratitis.[1] It is an often sight-threatening and difficult-to-treat infection because trophozoites (the multiplicative stages) may differentiate into cysts (resistant, dormant stage) in response to drugs that act by different mechanisms of action[2].

Abrasion of the cornea increases the exposure of mannose-containing glycoproteins, to which the amoebae can adhere with high affinity. Subsequently, trophozoites penetrate and destroy the corneal epithelium, perforate the Bowman's membrane, and enter the underlying stroma[1].

There are about 20 genotypes described in the genus *Acanthamoeba*, and strains from almost all of them are able to infect humans, causing *Acanthamoeba* keratitis, granulomatous amoebic encephalitis, and disseminated infections[3]. The T4 genotype is the most prevalent in the environment and most frequently associated with keratitis, suggesting that its pathogenic potential (greater capacity for binding and therefore greater cytotoxic capacity) is directly related to its genotypic characteristics[4].

Trophozoites and cysts are normally found in soil and water samples, and risk factors include exposure of contact lenses, lens case, or even the hands to these samples. These factors associated with the late diagnosis favor disease progression, sometimes leading to the need of corneal transplantation[1,4].

CASE REPORT

A 28-year-old female contact lens wearer reported red eye and foreign body sensation for one week. She sought medical attention and was diagnosed with keratitis. She initiated topical treatment with ciprofloxacin and dexamethasone eye drops. The patient followed the treatment protocol, without improvement. After one week of treatment, she came to our service at University Hospital of Brasília [*Hospital Universitário de Brasília* (HUB)], Brazil, complaining of severe ocular pain and intense conjunctival hyperemia. Furthermore, a whitish ring shaped corneal infiltrate was observed during clinical examination.

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The ophthalmologic examination revealed infectious keratitis with ring infiltrate, ciliary injection 3+/4 and conjunctival hyperemia 3+/4 (**Figure 1A, B** and **C**). Corneal scrapes and contact lens case solution were plated on non-nutrient agar seeded with inactivated *Escherichia coli* XL10-Gold, incubated at 26°C and 37°C. Total DNA was extracted using the Mini Spin Plus kit (BioPur, Pinhais, PR, Brazil) following the manufacturer's protocol, and PCR was performed for *Acanthamoeba* genus using specific primer pairs, JDP1 (5′-GGC CCA GAT CGT TTA CCG TGA A-3′) and JDP2 (5′-TCT CAC AAG CTG CTA GGG GAG TCA-3′)[5].

Owing to the clinical condition of the patient and a history of wearing contact lens without proper hygiene, including the use of non-sterile cleaning solutions and handling of the lens with wet hands, *Acanthamoeba* keratitis was suspected, and specific treatment with brolene and biguanide eye drops was initiated.

After three months of clinical treatment, the patient underwent an emergency penetrating keratoplasty (PKP) due to corneal opacification, with vision acuity of 20/20 after surgery. The procedures were conducted in accordance with the tenets of the Declaration of Helsinki. Approval of the study was obtained from the institutional review boards of the University of Brasília, Faculty of Medicine.

Acanthamoeba was isolated from two sources, i.e., corneal scraping and contact lens case, and was confirmed by PCR. Typical trophozoite morphology and double-walled cysts were detected in the culture media (**Figure 2A** and **B**). DNA fragments amplified with the *Acanthamoeba*-specific primer pairs JDP1 and JDP2 (**Figure 3A**) were sequenced, and the partial 18S rDNA sequences were compared with those of environmental *Acanthamoeba* isolates from Brasília, Brazil[6]. (GenBank) (**Figure 3B**). The results indicated high similarity of the cornea (BsB6) and contact lens case (CLC1) isolates (99% and 97%, respectively) with the T4 genotype. The sequences were deposited in the GenBank database (BsB6- MF164154 and CLC1- MF164153). In comparison with other environmental isolates from the patient's city of residence, a high similarity rate was observed (**Figure 3B**).

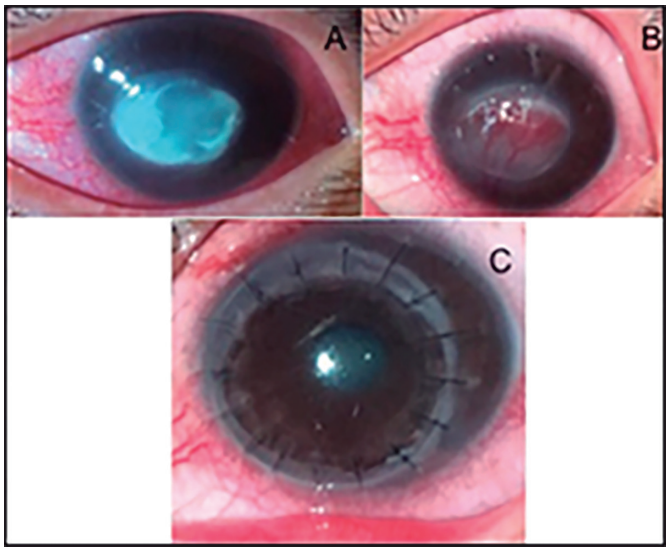

FIGURE 1: Different stages of *Acanthamoeba keratitis* in a patient from Brasília, Brazil. **A.** Stromal infiltrate at patient's admission **B.** Opacification with neovascularization upon treatment with brolene and biguanide eye drops **C.** Aspect of the cornea after PKP. **PKP**: penetrating keratoplasty.

DISCUSSION

Diagnosing *Acanthamoeba* keratitis is a major challenge, especially because the initial phase is similar to other diseases causing keratitis, such as herpes simplex. Furthermore, corticoid therapy usually leads to delayed anti-amoebic treatment, worsening the prognosis. In this particular case, the patient was treated with corticosteroids, which could have favored the progression of the disease. The incidence per year of

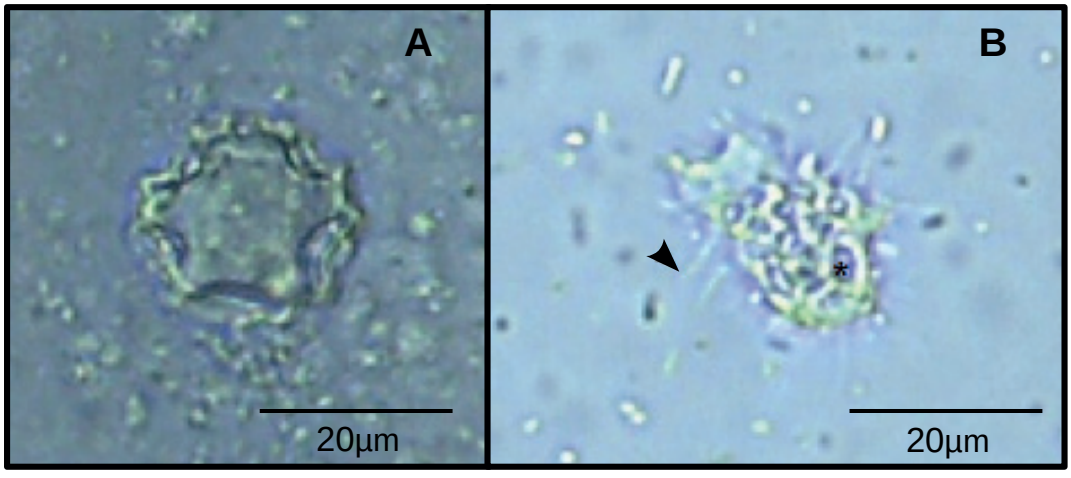

FIGURE 2: Microscopy images of corneal isolate of *Acanthamoeba*. **A.** Cyst and **B.** trophozoite from washed culture plates (containing non-nutrient agar with inactivated *Escherichia coli*) showing: ► acanthopodia; * contractile vacuoles (magnification, 1,000×).

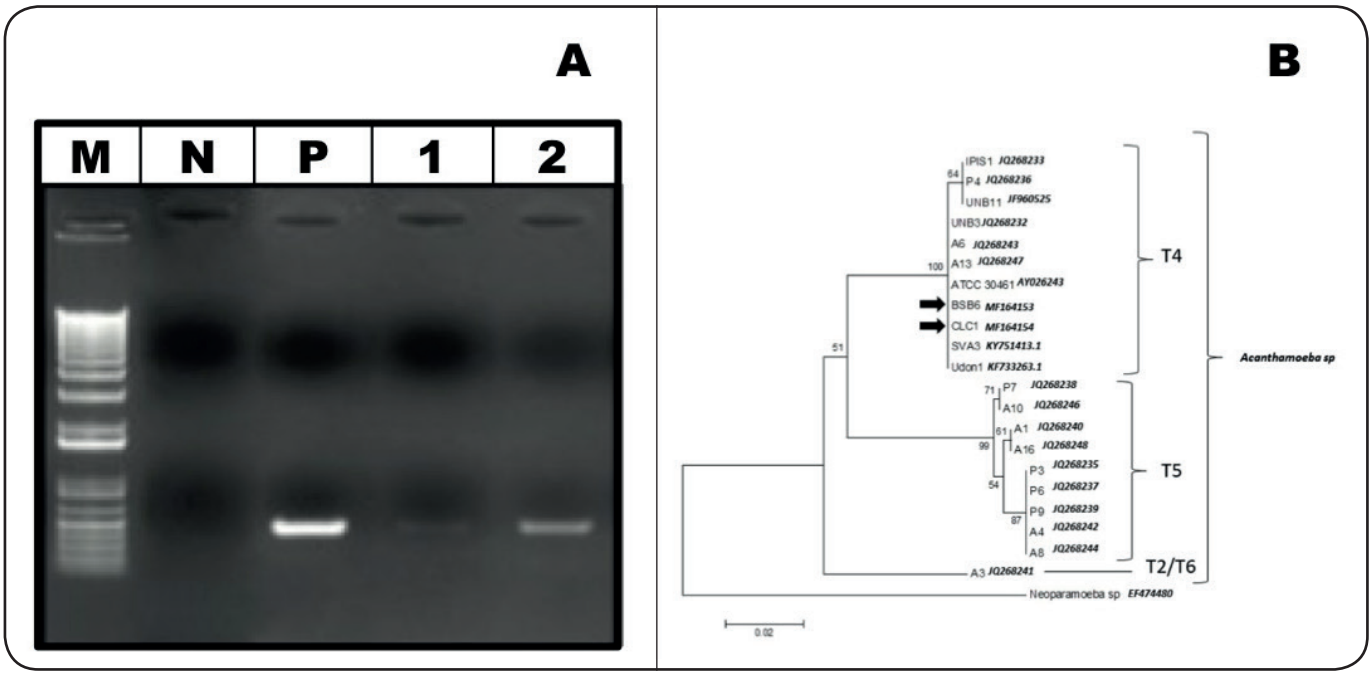

FIGURE 3: A. Agarose gel electrophoresis of DNA fragments amplified with the *Acanthamoeba*- specific primers JDP1 and JDP2. **M:** molecular size marker (1kb ladder plus); **N**: negative control (mix without DNA); **P:** positive control (ATCC 30461 reference strain - *Acanthamoeba polyphaga*); **1:** corneal isolate (BsB6); **2:** contact lens case isolate (CLC1). **B.** Phylogenetic relationships between *Neoparamoeba* sp. (outgroup) and 17 isolates of *Acanthamoeba* sp. inferred by comparison of partial 18S rRNA sequences The figure shows a neighbor-joining tree generated with the Kimura 2-parameter model. The GenBank reference code is shown next to the names of the isolates. The bootstrap values obtained from 1,000 repetitions are indicated near the nodes. The isolates analyzed in this study are indicated with black arrows (BSB6 MF164153 – corneal isolate and CLC1 MF164154 – contact lens isolate). Curly braces and black lines represent the genotype of each isolate (T4, T5, T2 / T6)[6]. Black arrows represent: **DNA:**deoxyribonucleic acid); **BsB6:** corneal isolate)**; CLC1:** contact lens case isolate.

Acanthamoeba keratitis among contact lens wearers in developed countries is increasing, which could be in part due to lens exposure to environmental contamination during care procedures[2]. The T4 genotype identified here is the most frequently reported genotype in clinical and environmental sources[3].

Although there are cases of keratitis caused by other free-living amoebae from the Vahlkampfiidae family or from the genus *Hartmannella*[4], SSU RNA amplification and sequencing may help to confirm clinical suspicions and results obtained from culture media. In an experimental study conducted in Brazil on corneal scraping and biopsies from an AK rat model, the authors evaluated two DNA extraction techniques and a semi-nested-PCR (snPCR) targeting the 18S rRNA gene to detect *Acanthamoeba* cysts and trophozoites. Similar to our findings, they reported higher positivity when comparing culture and PCR methods, thus demonstrating the importance of performing two diagnostic techniques for human keratitis[7].

Acanthamoeba keratitis cases have been described since 1988 in Brazil[8] mainly from the southern and southeast regions[9,10]. The indiscriminate sale of disposable gelatin contact lenses[11], and the importance of *Acanthamoeba* keratitis as a well-established clinical entity in ophthalmology have been discussed[12]. To the best of our knowledge, this is the first report of T4 *Acanthamoeba* keratitis case from the Midwest region of Brazil. The strict practice of sanitizing contact lenses, and of protecting them from environmental sources of contamination, along with early diagnosis of the disease should be promoted, in order to minimize the risk of infection by this protozoan.

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Conflict of interest

The authors declare that there is no conflict of interest.

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