Keratitis due to an Unusual Pathogenic Social Amoeba, *Dictyostelium* polycephalum

To the Editor—Infections with pathogenic free-living amoeba including *Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Sappinia pedata* have been documented in many countries [1]. However, social amoeba have not been reported earlier as etiological agents in human infections. In April 2010, we reported a case of keratitis in an immunocompetent person by social amoeba, *Dictyostelium polycephalum* [2]. In the present study, we report a second case of keratitis by *D. polycephalum*.

A 15-year-old, non–contact lens wearer presented with a 20-day history of pricking sensation, redness, swelling, pain, and watering in the right eye after injury with a fingernail. The right eye showed lid edema, the conjunctiva was congested, and the cornea showed a large epithelial defect with a whitish yellow infiltrate measuring 10 × 10 mm involving almost all the cornea (Figure 1A). Visual acuity was expressed as the ability to see hand movements near the face. Ultrasonography of the right eye showed an attached retina with an echo-free vitreous cavity. At the time of the visit, the patient was using 0.5% moxifloxacin eye drops installed 5×/d. Corneal scrapings were collected using a sterile surgical blade (#15 on a bard parker handle) under topical anesthesia. Corneal scrapings were subjected to direct microscopic examination and inoculated onto blood agar, brain-heart infusion broth, Sabourauds dextrose agar, and non-nutrient agar (NNA).

The direct microscopic examination of corneal scraping revealed double-walled

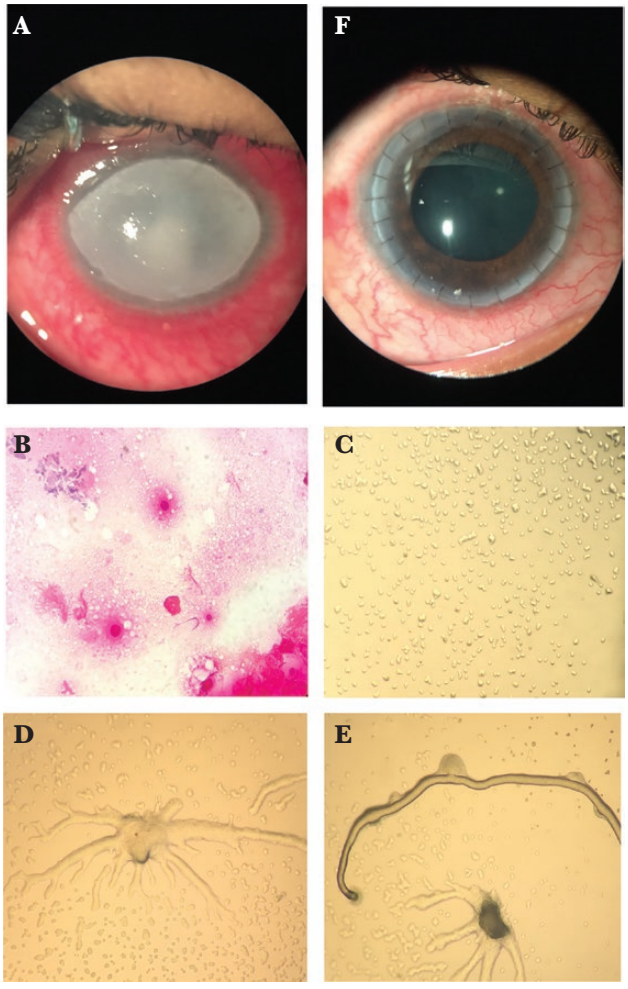

Figure 1. (A) Cornea of the patient's right eye showing whitish yellow infiltrate. (B) Spherical cysts morphologically resembling *Dictyostelium* species in Gram stain. (C) Unicellular myxamoeba of *Dictyostelium* on non-nutrient agar. (D) Cell streaming, wheel-shaped aggregates of myxamoeba of *Dictyostelium*. (E) Pseudo plasmodia (slug) of *Dictyostelium*. (F) Corneal graft of the patient at the last follow-up.

cysts morphologically resembling *Dictyostelium* species in potassium hydroxide mount, Gram stain (Figure 1B), and Giemsa stain. Based on direct microscopy findings, a presumptive diagnosis of *Dictyostelium* keratitis was made. As the size of the infiltrate was too large to treat medically and no data on antimicrobial susceptibility of *Dictyostelium* were available, surgical treatment was advised. Penetrating keratoplasty was performed instead of lamellar keratoplasty as the infiltrate had penetrated the descemet's membrane and there was a small endoexudate in the visual axis. Postoperatively, the patient was treated with topical 1% prednisolone acetate eye drops every 2 hours and 0.5% moxifloxacin eye drops every 2 hours. After a week, the antibiotic was stopped, and steroid eye drops tapered gradually.

Several unicellular myxamoeba (Figure 1C), cell streaming, wheel-shaped aggregates of myxamoeba (Figure 1D), pseudo plasmodia (slugs) (Figure 1E), and spherical cysts were seen after 48 hours of incubation on an NNA plate inoculated with corneal scraping. Fruiting bodies or Sorocorps that are very small, typically clustered to form coremiform fructifications, and variable in number were observed on NNA plates after 5 days of incubation. Based on morphology, the organism was identified as *Dictyostelium polycephalum*. The corneal button inoculated onto the NNA plate showed the growth of *Dictyostelium*. DNA was extracted from the growth on the NNA plate, inoculated with corneal scrapings and directly from the corneal button. The extracted DNA was subjected to 18S rDNA polymerase chain reaction (PCR) for free-living amoebas [3]. The PCR product was subjected to bidirectional sequencing, and the sequence is deposited at GenBank (GenBank accession number MF780724). The morphological identification of the organism was confirmed by sequencing. At the last follow-up (4 months after the surgery), the corneal graft of the patient was clear (Figure 1F), with no evidence of infection, and the best corrected visual acuity was expressed as 6/9.

The life cycle of *Dictyostelium* is unique, and the transition from unicellular organism to multicellular fruiting body is seen during the life cycle [4]. The vegetative stage of *Dictyostelium* species is a free-living myxamoeba. When the food becomes scarce, the myxamoeba aggregates to form a slug or pseudo plasmodium. The slug forms a fruiting body. Under some conditions, some myxamoeba enter into an encystment stage, termed a microcyst. The sexual life cycle of *Dictyostelium* species involves the formation of macrocysts. Instead of the myxamoeba forming a slug, two of the aggregating amoebae fuse, consume the remaining cells, and form a macrocyst [5, 6]. There are more than 100 species of *Dictyostelium* identified so far [5]. It is quite surprising that in both the cases of *Dictyostelium* keratitis the species identified is polycephalum. *Dictyostelium* species are found in arctic, tropical, desert, and rain forest soils [4]. There are a number of ecological changes that offer opportunities for existing nonpathogenic organisms to become pathogenic and adapt to the human body. A fingernail contaminated with soil may be the source of infection in the present patient.

The clinical presentation is similar in the 2 cases, except for the presence of endoexudates in the visual axis in the second patient. Both the patients were treated by surgery. However, there is a need to study the antimicrobial susceptibility of this emerging pathogenic organism for the medical management of patients.

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Study on human subjects. The procedures followed in the study were in accordance with the ethical standards of Helsinki declaration and the patient written consent obtained.

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