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Case Report: Imported *Pythium insidiosum* Keratitis After a Swim in Thailand by a Contact Lens-Wearing Traveler

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Abstract. A 30-year-old woman with a history of contact lens wear and exposure to swimming pool water in Thailand presented with a non-responsive, progressive corneal ulcer of the right eye. Confocal microscopy evidenced septate linear branching structures, raising suspicion of fungal keratitis. She was promptly treated with topical antibiotics and both topical and intravenous caspofungin plus voriconazole. Worsening of the clinical picture after 1 month of intensive medical therapy led to a large therapeutic penetrating keratoplasty being performed. Corneal cultures grew a mold-like organism, which was identified by sequencing as *Pythium insidiosum*, an aquatic oomycete. After 4 years of follow-up, the graft exhibits no infection relapse, but graft transparency has been lost after two rejection episodes. Keratoplasty combined with antifungal treatment may offer a cure to *P. insidiosum* keratitis, although long-term preservation of corneal transparency is difficult to obtain.

INTRODUCTION

Pythium is an aquatic fungal-like oomycete that lives in soil and water in tropical, subtropical, and temperate climates. Numerous *Pythium* species are plant pathogens; however, *P. insidiosum* and the recently recognized *P. aphanidermatum* are the only two members of the genus responsible for diseases in mammals, including humans.[1] *P. insidiosum* is the most frequently recovered species in the *Pythium* genus; it was first documented in 1985 in Thailand in two patients presenting with chronic granulomatous ulcers on the extremities.[2,3] Since that time, almost 150 cases of infection caused by this oomycete have been published in the literature, with high morbidity and mortality rates noted; the great majority occurs in Thailand, where the disease is widely distributed throughout the country and is considered endemic.[2,4,5] Indeed, *Pythium* species inhabit aquatic and moist soil environments, such as irrigation channels, household irrigation reservoirs, and rice paddies, the latter being very common in Thailand.[6] There are four different clinical forms of human pythiosis: the vascular form resulting in arterial occlusions or aneurysms; the ocular form causing corneal ulcer; the cutaneous/subcutaneous form presenting with granulomatous, ulcerating, or cellulitic lesions; and the disseminated form. These different forms account for 59%, 33%, 5%, and 3% of the cases, respectively, in the largest published case study of human pythiosis.[2] Except for the ocular form, most patients with pythiosis have underlying chronic hemolytic anemia, such as thalassemia-hemoglobinopathy or paroxysmal nocturnal hemoglobinuria.[2] Human pythiosis cases occur worldwide but are probably underrecognized and thus, misdiagnosed.[7,8] We report a case of contact lens-related *P. insidiosum* keratitis imported in France after traveling to Thailand, in which the strain was identified by nucleic acid sequencing and the patient was cured with a large therapeutic penetrating keratoplasty.

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CASE REPORT

A 30-year-old woman presented with pain and decreased vision in her right eye for 24 hours. She had a history of daily-wear soft contact lens use (Bausch & Lomb, Montpellier, France) for myopia without complications. The patient used to take out her lenses in the evening, and she cleaned her lenses with an all-in-one solution appropriately. Symptoms started on her way back from a trip to Thailand in April, where she swam in a swimming pool without removing her contact lenses. After she came back to France, a combination therapy based on topical dexamethasone and tobramycin was prescribed for a presumed bacterial corneal ulcer. Ten days later, the patient was referred to the French Quinze-Vingts National Eye Hospital because of rapid worsening of clinical features. On examination (day 1), the best corrected visual acuity was 20/200 in the right eye and 20/20 in the left eye. Fluorescein eye stain test and slit-lamp examination revealed severe conjunctival injection and a central corneal ulcer with subepithelial and superficial stromal infiltration in a reticular pattern with feathery edges, satellite lesions, and Wessely ring in the right eye (Figure 1A–C). Confocal microscopy evidenced septate linear branching structures resembling fungal filaments (Figure 1D). Corneal scrapings were performed for direct microscopic examination after May Grünwald Giemsa staining and culture on chocolate PolyViteX agar, Schaedler broth with globular extract, and Sabouraud with antibiotics agar. Because her corneal lesions were clinically compatible with fungal keratitis, she was prescribed hourly topical 1% voriconazole and 0.25% amphotericin B in addition to empirical antibiotic treatment (Bacitracin and Colimycin eye drops; one drop per hour). Oral voriconazole at a dosage of 200 mg two times per day was added at day 3 because of the lack of clinical improvement. No fungal element was evidenced by direct microscopic examination and culture from the initial corneal scraping. Grocott staining of the corneal biopsy performed at day 3 revealed large sparsely septate hyphae, suggesting a mold that failed to grow in culture. Polymerase chain reactions (PCRs) for herpesviruses and *Acanthamoeba* were negative, whereas bacterial cultures evidenced *Propionibacterium acnes* from corneal scraping and *Corynebacterium renale* and *Staphylococcus*

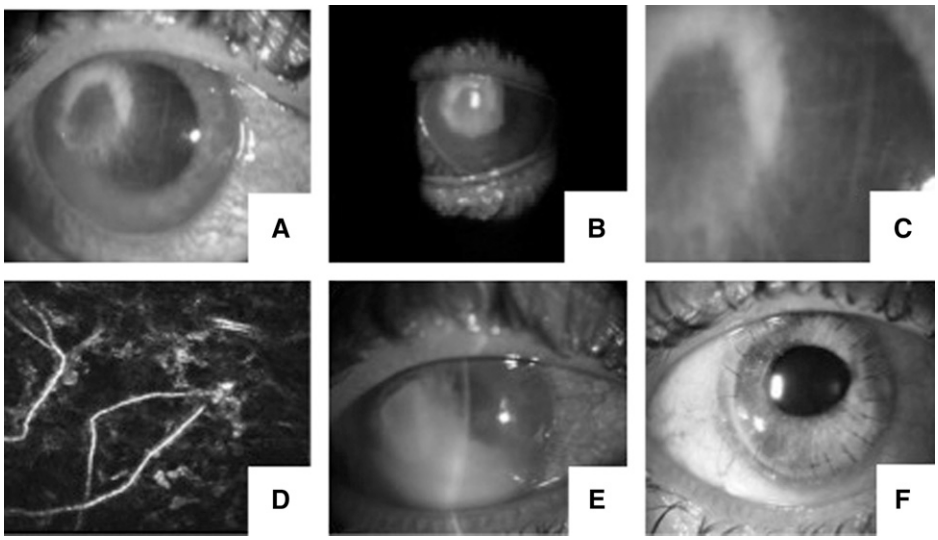

FIGURE 1. (**A**) Slit-lamp photograph and (**B**) fluorescein eye stain on admission showing a large central corneal ulcer with underlying dense stromal infiltrates. (**C**) Image magnification shows a reticular pattern of subepithelial and superficial stromal infiltration. (**D**) Confocal microscopy reveals highly reflective branching structures resembling fungal hyphae. (**E**) Worsening of clinical features at day 31. (**F**) Clinical photograph 6 months after the penetrating keratoplasty.

capitis from corneal biopsy. After a 14-day follow-up period, corneal infiltration progressively worsened and was accompanied by the onset of hypopyon and a severe decrease in her visual acuity from 20/2,000 to light perception. Thus, both topical and intravenous caspofungin were added to her regimen, and voriconazole was administered intravenously at a dosage of 300 mg two times per day. Intravenous imipenem associated with topical gentamicin, ticarcillin, and vancomycin was also introduced. Despite aggressive medical treatment, clinical deterioration led physicians to perform an intrastromal injection of voriconazole 30 days after the first examination (Figure 1E). After dilution in Ringer lacate (0.5 mg/mL), four intrastromal injections were performed in the peripheral corneal stroma (temporal, nasal, superior, and inferior parts of the cornea) with a 30-gauge needle (0.1 mL). Finally, an urgent large therapeutic penetrating keratoplasty was performed at day 38. Anterior chamber irrigation with 1% povidone iodine for 20 minutes followed by prolonged balanced salt solution irrigation was also carried out during keratoplasty. Pathologic examination of the host corneal showed an ulcerated epithelium, stromal edema with inflammatory infiltrate, and extensive loss of endothelium. The Gomori methenamine silver staining revealed numerous broad, branching hyphae within the anterior corneal stroma up to the pre-descemetic layer (Figure 2). A few days after surgery, corneal cultures grew an organism with sparsely septate,

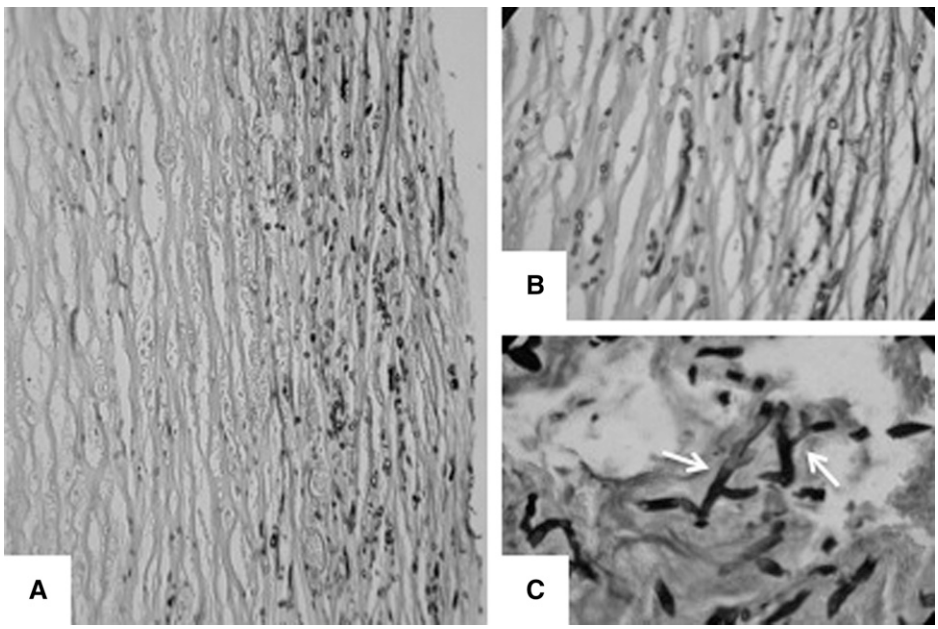

FIGURE 2. Histopathology of the excised host cornea. (**A**–**C**) Gomori methenamine silver revealing branching hyphae (arrows in **C**) within the anterior part of the corneal stroma.

broadly branching hyphae on standard Sabouraud's agar. The isolate was sent to the National Reference Center for Invasive Mycoses and Antifungals (NRCMA), Institut Pasteur, Paris, France for speciation and identified as *P. insidiosum* by amplification and sequencing of the D1/D2 region of the 28S subunit of ribosomal DNA.[9] Comparison of the nucleotide sequence of our isolate (777 base pairs) with the GenBank database revealed a 99.4% similarity (772 of 777 base pairs) with *P. insidiosum* collection strain CBS 574.85 (GenBank accession no. AY598637). Immediate post-operative treatment consisted of topical voriconazole, caspofungin, bacitracin, and rifamycin associated with 2% cyclosporine eye drops for prevention of corneal allograft rejection. Dexamethasone eye drops were introduced 2 weeks after transplantation when it became evident that the fungal infection was clinically cured (i.e., absence of stromal infiltrates and corneal ulcer). Post-operatively, slit-lamp examination showed the presence of aqueous cells at low level, which is consistent with normal post-keratoplasty anterior chamber reaction, absence of stromal infiltrates, and no graft vascularization. The anterior chamber reaction decreased and disappeared during the first 2 weeks after transplantation (Figure 1F). Topical voriconazole and caspofungin eye drops were discontinued 3 months after transplantation. Because the eye was at high risk of rejection, corticosteroid use was never stopped, and topical cyclosporine was progressively tapered and finally discontinued 18 months after transplantation. Two allograft rejection episodes occurred 12 and 30 months after transplantation. They were treated with intravenous methylprednisolone pulses (500 mg/24 hours) for 3 days. Currently, the patient is completing her fourth year of follow-up without signs of recurrent infection. Graft transparency did not recover after the last rejection episode, and visual acuity is limited to 20/2,000.

DISCUSSION

In total, 45 cases of *Pythium* keratitis have been reported from Thailand ($N = 39$),[2,10–12] Haiti ($N = 1$),[13] New Zealand ($N = 1$),[14] Australia ($N = 1$),[15] Israel ($N = 2$),[16,17] and Malaysia ($N = 1$),[18] and all cases, except for one patient returning from Israel, were indigenous cases.[17] In most cases, patients have a history of being recently exposed to wet environments (wastewater, rice paddies, swimming pool, tap water, or river) or farming occupations, which was shown in a series of 11 cases of *Pythium* keratitis in which 91% patients were farmers.[10] Recently, Thanathanee and others[11] reported an outbreak of four consecutive cases of ocular pythiosis during the rainy season in Thailand; all of them had a history of fungal keratitis after being exposed to contaminated water (wastewater, rice field [$N = 2$], or contaminated rainwater). In patients with ocular pythiosis, direct contact with the pathogen is likely an initial step of infection. Exposure to water while wearing contact lens may be a supplementary pre-disposing factor for *Pythium* keratitis. There are only four published reports of *Pythium* keratitis linking this infection to contact lens use, and all reported cases had a history of water contamination as well (river, tap water, and swimming pool).[12,16–18] Unlike *Pseudomonas aeruginosa* or *Acanthamoeba*, filamentous fungi were historically infrequently linked to contact lens-associated keratitis.[19–21] In 2005, a multicountry outbreak of contact lens-associated keratitis caused by the *Fusarium* species occurred and was suspected to be linked with the use of a specific brand of contact lens multipurpose solution that was contaminated.[22] Since that time, fungal keratitis associated with contact lens wear has been rising.[23,24] Currently, species from the *Fusarium*, *Aspergillus*, *Candida*, and *Curvularia* genuses are the most common causes of these keratitis. As described for these fungi, *P. insidiosum* could take advantage of a pre-existing epithelial defect because of abrasions caused by contaminated contact lenses to initiate infection.[25]

As in our case, *Pythium* keratitis is usually misdiagnosed as other fungal keratitis. Indeed, corneal features are similar (e.g., stromal infiltrate with a feathery margin), and confocal microscopy may show septate linear branching structures resembling fungal filaments.[25] Culture of corneal scrapings and corneal biopsy are useful diagnostic methods for pythiosis, because *P. insidiosum* usually grows very fast in conventional nutrient agar, such as the Sabouraud medium. However, this oomycete is often confused with mucoraceous fungi, because the sparsely septate, hyaline hyphae-like elements of *Pythium* species resemble the sparsely septate, hyaline hyphae seen in the Mucorales.[26] The use of molecular tools, such as internal transcribed spacer (ITS) or 28S sequencing, is, therefore, needed to confirm the identity of the organism.

In this case and all previously reported cases of *Pythium* keratitis, prompt medical treatment alone failed to cure the infection.[10–12,14,16–18,27] *P. insidiosum* poorly responds to conventional systemic or topical antifungal agents (i.e., amphotericin B and triazoles), because it lacks the drug target ergosterol in its cytoplasmic membrane. However, echinocandins, the antifungal agents that inhibit the β-glucan synthesis of the cell wall, may be effective in the treatment of pythiosis.[28] However, because of the aggressive and deeply invasive nature of *P. insidiosum*, surgical debridement of the infected tissue in association with antifungals seems to be the only effective treatment. The infection can progress deeply and cause endophthalmitis; however, the dissemination of *Pythium* from the cornea has not yet been recorded. In the case of deep extension, the ocular structures must be removed by enucleation or evisceration to cure the disease.[2,17] For localized disease, a penetrating keratoplasty should be performed, because it seems to be the only conservative treatment.[11,13,15,16,18] Nevertheless, infection can post-operatively recur if organisms are left behind.[10,12,14,17] In the largest series on *Pythium* keratitis, of 19 patients whose final outcome was known, 4 patients (21%) had eyes saved by anterior lamellar or penetrating keratoplasty, whereas 15 patients (79%) lost eyes after an enucleation or an evisceration that was performed to control the infection.[2] Recently, the efficacy of the immunotherapeutic vaccine based on a modified *P. insidiosum* antigen formulation has been reported for prevention of recurrence after keratoplasty in three cases of *Pythium* keratitis.[11] In all cases, patients received three doses of vaccine (100–200 μL, 2.0 mg/mL) at 2-week intervals after keratoplasty, whereas clinical worsening was detected after initial treatment with antifungal agents and full-thickness trephination with a scleral patch graft. Originally created for veterinary medicine, immunotherapy is increasingly used for human pythiosis with a moderate success rate.[29] It has been postulated that a switch in the host's immune response from a T helper 2 (Th2) (during infection) to a Th1 immune response (after vaccination) is behind the curative properties triggered by the vaccine.[30]

CONCLUSION

Our case shows a successful 4-year follow-up after a large penetrating keratoplasty was performed as a result of severe imported keratitis caused by *P. insidiosum* that worsened despite antifungal combination therapy. *P. insidiosum* may be the etiology of numerous unknown fungal etiologies of corneal ulcers in endemic areas. This oomycete should now be promptly suspected in the context of fungal keratitis occurring in a traveler returning from Thailand, because early penetrating keratoplasty associated with antifungal treatment can be required to cure ocular pythiosis.

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