Pythium Keratitis

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12.1 Introduction

Human pythiosis is fatal, and an emerging disease of the tropical [1], subtropical, and temperate region. Although more commonly reported from southeast Asia [1, 2] (i.e., India, Indonesia, Japan, Korea, New Guinea, and Thailand), it has also been reported from the eastern coast of Australia and New Zealand, South America (Argentina, Brazil, Colombia, Venezuela), Costa Rica, Guatemala, Haiti, Panama and Nicaragua, and North America (Mexico, the United States, and Gulf coast states) [1, 3]. It is caused by a fungus like, oomycete *Pythium insidiosum* [4]. Human pythiosis [1] can be divided into four various clinical groups depending on their presentations: cutaneous/subcutaneous (5%), vascular (59%), ocular (33%), and disseminated cases (3%). Among the risk factors, Thalassemia-hemoglobinopathy syndrome [5] was found to be associated in most of the cases with cutaneous/subcutaneous, vascular, and disseminated pythiosis (85%). Ocular pythiosis is common during rainy season [2, 6], and the risk factors [1] include exposure to farming, direct contact with water resource (i.e., lake, river, lagoon, swamp, or even swimming pool), and contact lens wear [7–9]. Systemic immunosuppressants [10] used for chronic diseases like Crohn's disease have also been reported to predispose to such serious infections. Among the ocular involvement, corneal involvement was first reported by Vergille et al. in 1993 [11].

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S. Das, V. Jhanji (eds.), *Infections of the Cornea and Conjunctiva*,
https://doi.org/10.1007/978-981-15-8811-2_12

12.2 The Organism

Pythium is an oomycete which closely mimics fungus in its morphological features. It belongs to the genus of parasitic oomycetes. Previously they were classified under the fungi. It is primarily a plant pathogen but can cause pythiosis in animals. This was described as fungus in 1884 by Smith et al. [12] Due to lack of sporulation, it could not be identified until 1961, when it was first recognized as Hyphomycosis destruens by Bridges et al. [13] Later in 1974, Austwick and Copland [14] observed zoospore formation during transfer of colonies from Sabouraud's dextrose medium to aqueous medium. It has coenocytic hyphae [4] without septations. The term coenocytic refers to multinucleated cells without cytokinesis; this is caused by divisions of nucleus. Phylogenetically, it resembles more with algae than fungi. *Pythium* species belong to the kingdom *Stramenopila*, the class Oomycetes, the order *Pythiales*, and the family *Pythiacae*. It is not a true fungus. The cell wall [4] of *Pythium* lacks chitin, and has cellulose instead. The Cell membrane does not contain ergosterol. Production of zoospores is responsible for infection. Zoospores are single nucleated cells and biflagellate without a cell wall. It is considered to be an infective propagule which can swim and has chemotactic properties. When in contact with injured plants, zoospores encyst themselves and attaches to the plant via glycoprotein. It does not need any mammalian host for its survival as it can remain alive in its natural location. The life cycle as proposed by Mendoza et al., based on the light and electron microscopic features [15], is different in humans and plants. After colonization of plants, differentiation and maturation of sporangium occur with the release of zoospores which can swim and infect other plants. In humans or other animals after contact, zoospores lose their flagella and attach to the tissue with the help of a sticky substance followed by encystment, germination, and invasion resulting in pythiosis.

12.3 Clinical Features

The onset is usually 6–7 days with a history of exposure of any of the mentioned risk factors. The clinical features [6, 16, 17] at presentation usually include central white infiltration with tentacles like extension and pinhead lesions into the surrounding stroma (Figs. 12.1, 12.2, and 12.3). The infiltrate may be associated with

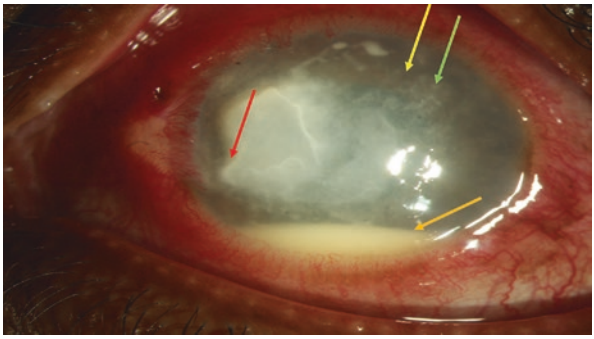

Fig. 12.1 Raised whitish infiltrate like a plaque (red arrow), tentacles (yellow arrow), pinhead lesions (green arrow), and hypopyon (orange arrow)

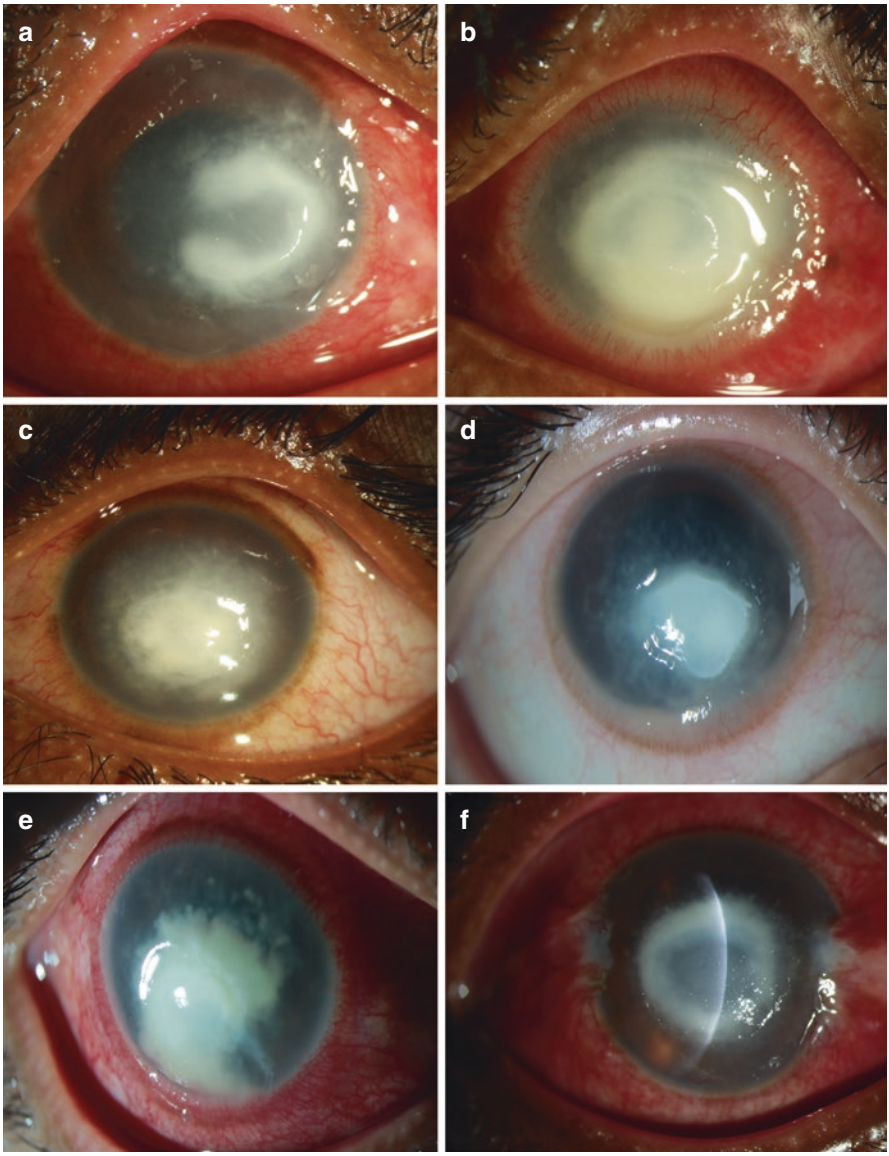

Fig. 12.2 Different presentations of *Pythium* keratitis: (**a**) peripheral ring shaped infiltrate with extension, (**b**) diffuse anterior to midstromal infiltrate, (**c**) central yellowish infiltrate with deep stromal extension, (**d**) raised whitish plaque with tentacular extension, (**e**) aggressive posterior stromal extension, and (**f**) ring shaped infiltrate with tentacle like extension

peripheral furrowing along with raised edges. Stromal thinning and deep vessels increase as the disease progresses. The frequency of each clinical sign at the time of presentation to clinic is shown in Table 12.1 [18]. The most important and frequent clinical sign associated is the presence of fine radiating tentacles along the margin of the infiltrate. However, there can be typical and atypical presentations (Fig. 12.2).

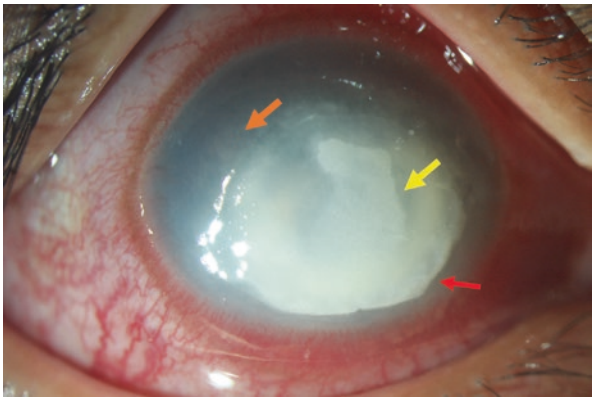

Fig. 12.3 Peripheral guttering (red arrow) with raised central plaque (yellow arrow) along with peripheral radiating tentacles (orange arrow) is uniformly present

Table 12.1 Clinical signs in cases of *Pythium* keratitis (some patients have more than one clinical sign)

Sl. #	Characteristics	%
(a)	Tentacles	86.96%
(b)	Pinhead lesions	82.61%
(c)	Guttering	46.38%
(d)	Hypopyon	46.38%
(e)	Plaque	20.29%
(f)	Central thinning	13%
(g)	Endoexudates	10.14%
(h)	Endothelial ring	2.90%
(i)	Perforated ulcer	1.45%

12.4 Diagnosis

Microbiological workup to confirm the clinical diagnosis includes corneal scraping of the infiltrate for direct microscopic examination of the *Pythium* filaments and/or its growth on culture media. These filaments are classically aseptate or sparsely septate, relatively broad, ribbon-like filaments as observed under direct microscopy on 10% potassium hydroxide with 0.1% calcofluor white or Gram stain. As illustrated by Mittal [19] et al., these filaments can be differentiated from fungal filaments with the help of potassium iodide–sulfuric acid (IKI-H_2SO_4). Growth on blood agar is flat, feathery edged, appressed, glabrous, colorless, or light brown radiating colonies (Fig. 12.4).

Formation of characteristic zoospores in aqueous medium, as described by Mendoza [15] et al., has been one of the measures to confirm the diagnosis [16, 20, 21] for *Pythium insidiosum*. Among oomycetes, formation of zoospores is also seen in *Phytophthora* and *Lagenidium*.

Other ways to diagnose are molecular methods such as PCR-based DNA sequencing [20, 22, 23] which have been used to confirm the species. Confocal microscopy has been used for in vivo diagnosis but could not differentiate conclusively from fungal keratitis [24].

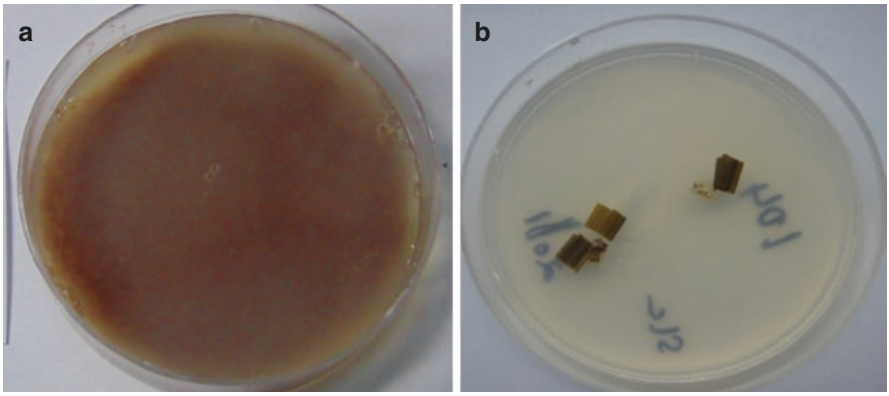

Fig. 12.4 Greyish, translucent, flat, and appressed growth with feathery edges on chocolate agar: (**a**) carnation leaves in an induction medium and (**b**) used for zoospore induction

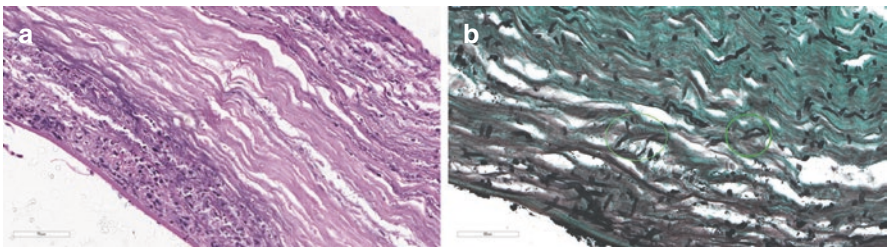

Fig. 12.5 Corneal histopathology of a corneal button of *Pythium* keratitis retrieved after therapeutic keratoplasty (**a**) Hematoxylin-Eosin stain (10×) and (**b**) GMS stain (20×). (Courtesy: Saumya Jakati)

On histopathological examination [19] of the corneal button obtained after keratoplasty, an inverse correlation was observed between the degree of inflammatory infiltrates and load of *Pythium* filaments (Fig. 12.5). Granulomatous inflammation was found in 30% of corneal tissue from therapeutic keratoplasty (TPK) specimens of *Pythium*. Other important findings were a relatively rare invasion of Descemet's membrane in contrast to fungal keratitis where DM perforation by fungal filaments are more commonly observed. Involvement of other ocular structures has been found to be less common. On electron microscopy, these hyphae were found to have a layered zone separating cell wall and membrane, but the most important finding was the presence of vesicles, which were either empty or filled with homogenous material [25].

12.5 Treatment

Medical treatment of *Pythium* keratitis alone may not be sufficient to manage the keratitis. Therapeutic keratoplasty may be required in some cases. Some of the causes include delayed or inaccurate diagnosis due to close similarity with fungal

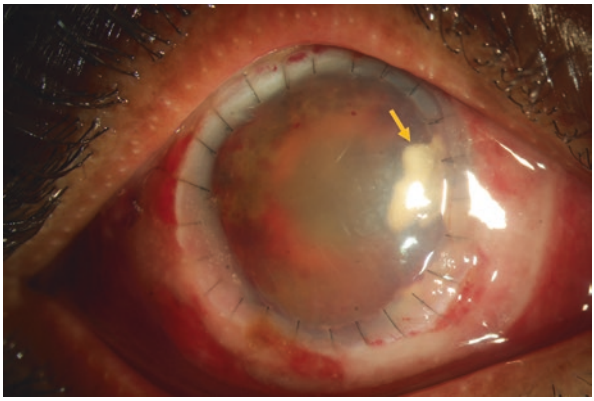

Fig. 12.6 Recurrence of the *Pythium* infection at the deep stromal level near graft host junction

filaments. In the recently published prospective trial by Permpalung [26] et al., nearly half the number of patients had undergone enucleation due to worsening of infection and suggested early surgical intervention in terms of therapeutic keratoplasty. Similarly, Agarwal [17] et al. also reported the role of surgical management, as in their experience, medical treatment alone is not successful. They have also mentioned high risk of recurrences of *Pythium* keratitis after TPK (Fig. 12.6) and recommended adjuvant treatment in the form of cryotherapy to decrease the recurrence. There has been recent evidence related to the antipythium efficacy of alcohol [27].

Early diagnosis by keen observation of salient clinical and microbiological features can lead to proper diagnosis, and thereby modifying the prognosis. Based on in vitro susceptibility testing for antibiotics, and a pilot trial [28], the efficacy of combination of linezolid and azithromycin has been found to be effective in the treatment of *Pythium* keratitis. The Recommended regimen is to use topical linezolid 0.2% (prepared from IV preparation) and azithromycin eye ointment 1% along with oral azithromycin 500 mg once a day for nearly 2 weeks. Use of oral azithromycin needs preevaluation by a general physician to rule out any associated cardiac contraindication or possible interaction with any of the drug which may cause QT-interval prolongation in ECG. Based on this trial, it is advisable to initiate treatment in a strategic manner [18]. Firstly, it should be diagnosed with the help of clinical and microbiological examination. Then it should be confirmed by the formation of zoospores or PCR-assisted diagnosis. Cases who are not in very advanced stage of keratitis should be managed initially with combination of antibiotics with minimum of 2 weeks of treatment to ascertain the clinical response. Patients who show poor response with medical treatment manifesting as extension of the infiltrate either peripherally or posteriorly should be subjected to therapeutic penetrating keratoplasty with 1 mm of clear cornea as safe margin. Response to medical treatment is noted by blunting of the tentacles and clearing of the surrounding stroma (Fig. 12.7). On follow-up, a formation of peripheral thinning surrounding the infiltrate can be observed (Fig. 12.3). In cases with significant corneal thinning, cyanoacrylate glue application should be planned to avoid corneal perforation. In general,

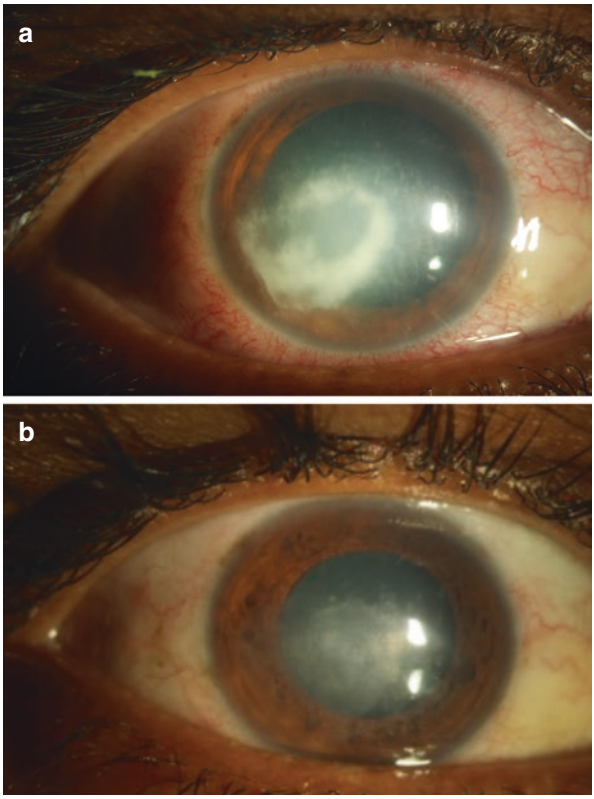

Fig. 12.7 (**a**) Whitish ring shaped infiltrate: (a) with surrounding tentacular extension with stromal edema which responded and (**b**) infiltrate responded to combination of antibiotics

infection resolves with increase in stromal vascularization associated with decrease in the size of infiltrate (Fig. 12.4). Medical treatment results in the resolution of infection in nearly 50–60% cases, provided early diagnosis of infection is made. Median duration of resolution is 3 months. The outcome of penetrating keratoplasty for corneal scar after resolution of keratitis is shown to be better as compared to active keratitis (Fig. 12.8).

12.6 Prognosis

The prognosis of infection depends on the early diagnosis. On reevaluations of the cases being managed by medical and surgical treatment, few prognostic factors have been noted. Factors associated with better prognosis are younger age at presentation without associated comorbid conditions, early confirmation of the diagnosis along with <6 mm size of the infiltrate, and limited to the anterior stroma. Cases with corneal Infiltrate extending to posterior stromal involvement with associated endo-exudates are usually associated with bad prognosis and need early surgical intervention. The tell-tale signs mentioned can help in providing strategic approach towards the management of *Pythium* keratitis.

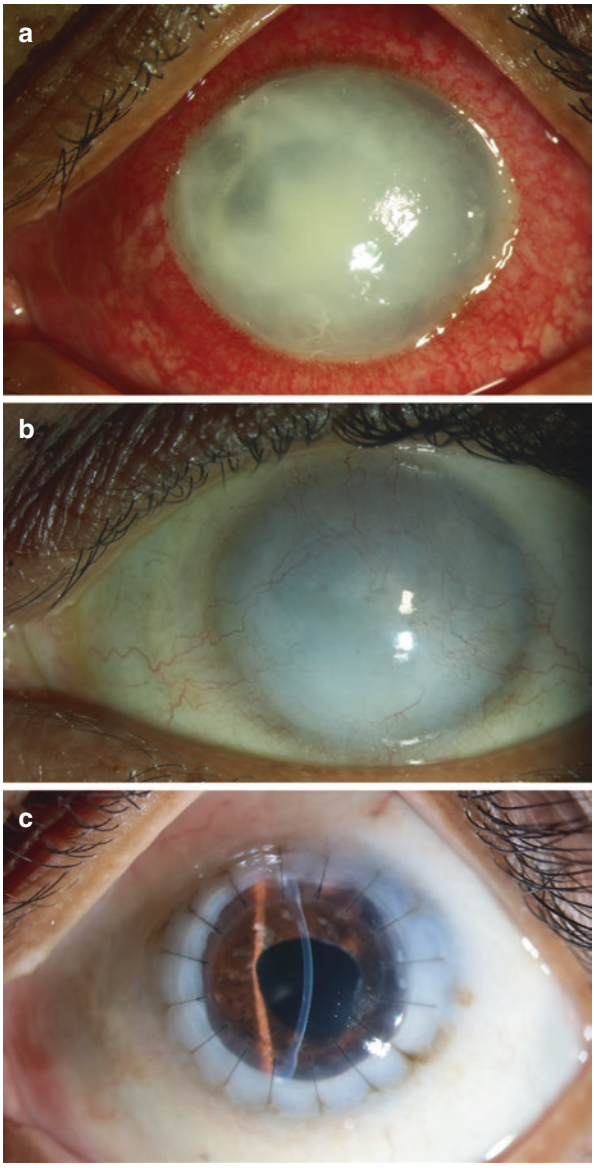

Fig. 12.8 Severe *Pythium* keratitis: (**a**) with lesions extending both posteriorly as well as till the limbus, (**b**) showing the complete resolution of infection following medical treatment, and (**c**) followed by optical keratoplasty along with cataract surgery with PCIOL placement

References

1. Krajaejun T, Sathapatayavongs B, Pracharktam R, Nitiyanant P, Leelachaikul P, Wanachiwanawin W, Chaiprasert A, Assanasen P, Saipetch M, Mootsikapun P, Chetchotisakd P, Lekhakula A, Mitarnun W, Kalnauwakul S, Supparatpinyo K, Chaiwarith R, Chiewchanvit S, Tananuvat N, Srisiri S, Suankratay C, Kulwichit W, Wongsaisuwan M, Somkaew S. Clinical and epidemiological analyses of human pythiosis in Thailand. Clin Infect Dis. 2006;43(5):569–76.

2. Thanathanee O, Enkvetchakul O, Rangsin R, Waraasawapati S, Samerpitak K, Suwan-apichon O. Outbreak of Pythium keratitis during rainy season: a case series. Cornea. 2013;32(2):199–204.
3. Presser JW, Goss EM. Environmental sampling reveals that Pythium insidiosum is ubiquitous and genetically diverse in North Central Florida. Med Mycol. 2015;53(7):674–83.
4. De Cock AW, Mendoza L, Padhye AA, Ajello L, Kaufman L. Pythium insidiosum sp. nov., the etiologic agent of pythiosis. J Clin Microbiol. 1987;25(2):344–9.
5. Chitasombat MN, Jongkhajornpong P, Lekhanont K, Krajaejun T. Recent update in diagnosis and treatment of human pythiosis. Peer J. 2020;8:e8555.
6. Hasika R, Lalitha P, Radhakrishnan N, Rameshkumar G, Prajna NV, Srinivasan M. Pythium keratitis in South India: incidence, clinical profile, management, and treatment recommendation. Indian J Ophthalmol. 2019;67(1):42–7.
7. Lekhanont K, Chuckpaiwong V, Chongtrakool P, Aroonroch R, Vongthongsri A. Pythium insidiosum keratitis in contact lens wear: a case report. Cornea. 2009;28(10):1173–7.
8. Tanhehco TY, Stacy RC, Mendoza L, Durand ML, Jakobiec FA, Colby KA. Pythium insidiosum keratitis in Israel. Eye Contact Lens. 2011;37(2):96–8.
9. Raghavan A, Bellamkonda P, Mendoza L, Rammohan R. Pythium insidiosum and Acanthamoeba keratitis in a contact lens user. BMJ Case Rep. 2018;11(1).
10. Hung C, Leddin D. Keratitis caused by Pythium insidiosum in an immunosuppressed patient with Crohn's disease. Clin Gastroenterol Hepatol. 2014;12(10):A21–2.
11. Virgile R, Perry HD, Pardanani B, Szabo K, Rahn EK, Stone J, Salkin I, Dixon DM. Human infectious corneal ulcer caused by Pythium insidiosum. Cornea. 1993;12(1):81–3.
12. Smith F. The pathology of bursattee. Vet J. 1884;19:16–7.
13. Bridges CH, Emmons CW. A phycomycosis of horses caused by Hyphomyces destruens. J Am Vet Med Assoc. 1961;138:579–89.
14. Austwick PK, Copland JW. Swamp cancer. Nature. 1974;250(461):84.
15. Mendoza L, Hernandez F, Ajello L. Life cycle of the human and animal oomycete pathogen Pythium insidiosum. J Clin Microbiol. 1993;31(11):2967–73.
16. Sharma S, Balne PK, Motukupally SR, Das S, Garg P, Sahu SK, Arunasri K, Manjulatha K, Mishra DK, Shivaji S. Pythium insidiosum keratitis: clinical profile and role of DNA sequencing and zoospore formation in diagnosis. Cornea. 2015;34(4):438–42.
17. Agarwal S, Iyer G, Srinivasan B, Agarwal M, Panchalam Sampath Kumar S, Therese LK. Clinical profile of Pythium keratitis: perioperative measures to reduce risk of recurrence. Br J Ophthalmol. 2018;102(2):153–7.
18. Bagga B, Kate A, Mohamed A, Sharma S, Das S, Mitra S. Successful strategic management of Pythium insidiosum keratitis with antibiotics. Ophthalmology 2020; Published online ahead of print.
19. Mittal R, Jena SK, Desai A, Agarwal S. Pythium insidiosum keratitis: histopathology and rapid novel diagnostic staining technique. Cornea. 2017;36(9):1124–32.
20. Badenoch PR, Coster DJ, Wetherall BL, Brettig HT, Rozenbilds MA, Drenth A, Wagels G. Pythium insidiosum keratitis confirmed by DNA sequence analysis. Br J Ophthalmol. 2001;85(4):502–3.
21. Thongsri Y, Wonglakorn L, Chaiprasert A, Svobodova L, Hamal P, Pakarasang M, Prariyachatigul C. Evaluation for the clinical diagnosis of Pythium insidiosum using a single-tube nested PCR. Mycopathologia. 2013;176(5-6):369–76.
22. Kosrirukvongs P, Chaiprasert A, Uiprasertkul M, Chongcharoen M, Banyong R, Krajaejun T, Wanachiwanawin W. Evaluation of nested pcr technique for detection of Pythium insidiosum in pathological specimens from patients with suspected fungal keratitis. Southeast Asian J Trop Med Public Health. 2014;45(1):167–73.
23. Kosrirukvongs P, Chaiprasert A, Canyuk C, Wanachiwanawin W. Comparison of nested PCR and culture identification of Pythium insidiosum in patients with Pythium keratitis. J Med Assoc Thail. 2016;99(9):1033–8.
24. Anutarapongpan O, Thanathanee O, Worrawitchawong J, Suwan-Apichon O. Role of confocal microscopy in the diagnosis of Pythium insidiosum keratitis. Cornea. 2018;37(2):156–61.

25. Badenoch PR, Mills RA, Chang JH, Sadlon TA, Klebe S, Coster DJ. Pythium insidiosum keratitis in an Australian child. Clin Exp Ophthalmol. 2009;37(8):806–9.
26. Permpalung N, Worasilchai N, Manothummetha K, Torvorapanit P, Ratanawongphaibul K, Chuleerarux N, Plongla R, Chindamporn A. Clinical outcomes in ocular Pythiosis patients treated with a combination therapy protocol in Thailand: a prospective study. Med Mycol. 2019;57(8):923–8.
27. Agarwal S, Srinivasan B, Janakiraman N, Therese LK, Kumar SK, Patel N, Thenmozhi V, Iyer G. Role of topical ethanol in the treatment of Pythium insidiosum keratitis—a proof of concept. Cornea 2020; Published online ahead of print.
28. Bagga B, Sharma S, Madhuri Guda SJ, Nagpal R, Joseph J, Manjulatha K, Mohamed A, Garg P. Leap forward in the treatment of Pythium insidiosum keratitis. Br J Ophthalmol. 2018;102(12):1629–33.