

Pythium insidiosum: report of the first case of human infection in Brazil*

Pythium insidiosum: relato do primeiro caso de infecção humana no Brasil*

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Abstract: Pythiosis is caused by an aquatic fungus-like organism, *Pythium insidiosum*, pathogenic to men and animals. A patient with a phagedenic ulcer on the leg is reported. Histopathological examination was suggestive of zygomycosis, response to antifungal drugs was poor and cure was obtained by means of wide surgical excision. Etiologic diagnosis was confirmed by molecular amplification and DNA sequencing of colonies isolated in Sabouraud agar. After BLAST analysis, the sequence showed 100% identity with those of *P. insidiosum* deposited on the GenBank.

Keywords: Brazil; Humans; *Pythium*; Skin ulcer

Resumo: A pitiose é causada por microorganismo aquático, fungo-símile, o *Pythium insidiosum*, patógeno de homens e animais. Observou-se um paciente com úlcera fagedênica no membro inferior, com exame anatomopatológico sugestivo de zigomicose, pouco sensível à terapêutica antifúngica, obtendo-se cura por meio de ampla exérese. A comprovação etiológica resultou de métodos moleculares, com amplificação e seqüenciamento de DNA de organismo isolado em ágar Sabouraud, observando-se 100% de analogia com seqüências de *P. insidiosum* depositadas no GenBank.

Palavras-chave: Brasil; Humanos; *Pythium*; Úlcera cutânea

Pythiosis is an infectious condition located cutaneous-subcutaneously, but is occasionally systemic, which occurs in men and animals, particularly equines.¹ It occurs mainly in tropical and subtropical regions, and is caused by the aquatic fungus-like microorganism *Pythium insidiosum* (kingdom Straminipila, phylum Oomycota, class Oomycetes).² The first human cases were reported in 1985, in

Thailand, and corresponded to patients who had chronic ulcers located in the lower limbs.³ Only 32 human cases have been published ever since. Their predominant clinical manifestation was systemic, with arterial compromising in 17 cases, ocular in nine, subcutaneous in five and cardiopulmonary in a single case.³ Predominance of Thailand as country of origin of reported cases is noteworthy, namely, 78%

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(25 out of 32), the others coming from the United States of America (two), Australia (two), New Zealand, Haiti and Malaysia (one case each). Evolution is usually severe, with a death rate of 47% among those with vascular affection, expressed, in general, as extremity necrosis and chronic cutaneous ulcers.³

The present report is about a 49-year-old male patient, public servant, coming from Paraguaçu Paulista, SP, with the complaint of a cutaneous ulcer on left leg for three months. Lesion began as a pustule, one week after having gone fishing in a lake of backwater, in which he remained with his legs submerged. Initial diagnosis was cellulitis.

Because he did not respond to antibiotic treatment, a skin biopsy was performed, whose anatomopathological examination revealed a microorganism with non-septated hyphae, compatible with "zygomycosis". Treatment with amphoterecin B was then instituted, and debridement of the lesion was carried out. Due to a rapid degradation of renal function, with an accumulated dose of 575mg, and worsening of the clinical condition, the patient was referred to the Department of Dermatology at Unesp (Botucatu, SP).

Upon hospital admission, he had a good general state, presented a single ulcerated lesion, measuring 20 cm in its largest diameter, grossly granulated bed, with seropurulent secretion and infiltrated erythematic-violet borders, located on the right pre-tibial region (Figure 1A). Laboratorial investigation revealed anemia, high levels of creatinine and blood urea nitrogen, hypokalemia, normal blood glucose and negative serology for HIV. A spindle-shaped biopsy of

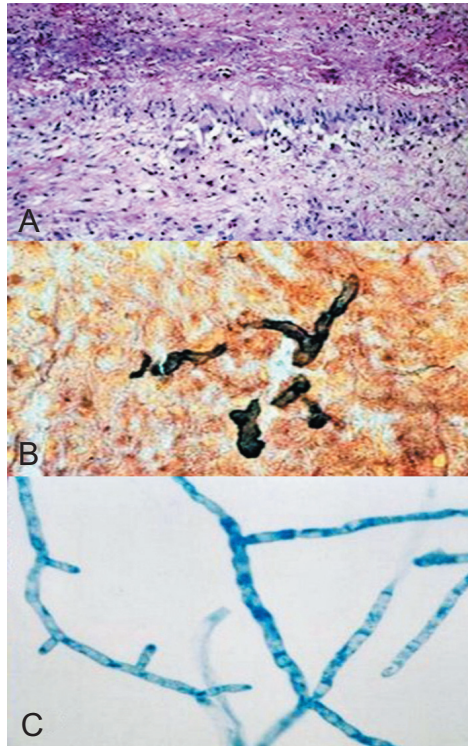


FIGURE 2: Anatomopathological examination showing (A) granuloma with palisade-like arrangement (HE 200x), (B) wide, distorted, septated hyphae, with peripheral reinforcement and 90° angle (silver 400x), (C) non-septated hyaline filaments, in agar-potato-dextrose (cotton blue 40x)

the lesion was performed, which revealed, upon anatomopathological examination, a chronic granulomatous process with rare silver-stained structures, compatible to the diagnosis of zygomycosis (Figures 2A and 2B). Countless attempts were made to culture in Sabouraud agar dextrose (SAD) medium and Mycosel®, all yielding negative results. Itraconazol at 400 mg/day was the proposed therapy, and an initial improvement was observed, with regression of the ulcer, followed, nevertheless, by the appearing of new tumors (Figure 1B). Potassium iodide, 4g/day, was associated, with no improvement. After three months of treatment, with progression of the lesion and no definitive diagnosis, the decision was for wide excision after previous tomographic delimitation. Itraconazol was substituted by amphoterecin B 15 days before surgical procedure, having been administered up to an accumulated dose of 1,800 mg.

With apparent resolution and granulation of the surgical bed, we proceed to grafting, with proper results in cosmetic quality, and apparent cure after a 24-month follow-up (Figure 1C). Tissue fragments, products of the excision, were seeded in SAD and agar potato dextrose, resulting in growth of a filamentous colony of membranous aspect, with large caliber hyphae on microscopy, and no conidia formation (Figure 2C). Molecular investigation was then carried out,⁴ with extraction of DNA and amplification of the ITS/5,8S rDNA region by the primers ITS4 and ITS5, according to White et al.,⁵ followed by visualization in



FIGURE 1: Phagedenic ulcer (A) with grossly granulated bed, purulent secretion, located on the right pre-tibial region; (B) after treatment with itraconazol, partial resolution of the ulcerated ulcer, nodules and nodosities on the proximal region; (C) six months after excision and grafting, displaying apparent cure

agarose gel, purification in GFX column (Amersham Biosciences) and DNA sequencing in ABI prism equipment, model 377 (Applied Biosystem, Foster City, Ca, USA). Sequences were aligned in the software *Clustal W* and then underwent analysis by (Basic Local Alignment Search Tool) (<http://www.ncbi.nlm.gov/BLAST>), comparing the obtained sequence with those deposited on GenBank and other databases. After analysis, sequence from the present case showed 100% identity with registered sequences of *P. insidiosum*, with full annealing of bases 253 to 845, which includes almost the entire gene 5.8S and region ITS2.⁴

Diagnosis of human pythiosis in this patient, yet extraordinary, is coherent with the frequency of pythiosis cases described in equines and sheep, or observed in dogs,^{*} both in Brazil^{6,7} and other countries.^{8,9} The possibility of diagnostic confusion with zygomycosis should be highlighted, given the histological similarities observed in the present case and described in the literature.¹⁰ Due to lack of ergosterol in the cytoplasmatic membrane of *P. insidiosum*, anti-fungal drugs are of little effectiveness in treatment, which makes surgical approach and immunotherapy the main therapeutic options.¹ □

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