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Surgical management and immunotherapy of *Pythium insidiosum* in a horse: case report

Inmunoterapia y manejo quirúrgico de *Pythium insidiosum* en un equino: reporte de caso

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ABSTRACT

Equine cutaneous pythiosis is caused by the oomycete *Pythium insidiosum*, which generally produces rapidly progressive granulomatous ulcerative lesions on the distal parts of limbs and abdomen. It is a globally distributed disease with higher prevalence in tropical and subtropical regions. The objective of this case report was to describe granulomatous lesions with necrotic tissue and fibrin bloody exudate in the palpebral region of the left eye caused by *Pythium insidiosum* in a quarter horse mare in Veracruz State, Mexico. The diagnosis was based on the clinical characteristics of the lesion and histopathology. Due to the extent of lesions, surgical resection was performed in combination with immunotherapy. Treatment with immunotherapy proved to be an effective alternative to achieve complete resolution of skin lesions.

Keywords: *Pythium insidiosum*, pythiosis, equine, immunotherapy.

RESUMEN

La pitiosis cutánea equina es causada por el oomiceto *Pythium insidiosum*, el cual generalmente produce lesiones ulcerativas granulomatosas de progresión rápida en las partes distales de las extremidades y del abdomen. Es una enfermedad distribuida globalmente y con mayor prevalencia en regiones tropicales y subtropicales. El objetivo de este reporte de caso fue describir las lesiones granulomatosas, con tejido necrótico y exudado fibrinosanguinolento en la región palpebral del ojo izquierdo ocasionadas por *Pythium insidiosum* en una yegua cuarto de milla en el estado de Veracruz, México. El diagnóstico se fundamentó en las características clínicas de la lesión y la histopatología. Debido a la extensión de las lesiones, se realizó la resección quirúrgica en combinación con la inmunoterapia. El tratamiento con la inmunoterapia demostró ser una alternativa eficaz para lograr la resolución completa de las lesiones cutáneas.

Palabras clave: *Pythium insidiosum*, pitiosis, equino, inmunoterapia.



INTRODUCTION

The etiological agent *Pythium insidiosum* (*P. insidiosum*) belongs to the class *Oomycetes*, whose zoospores lack chitin and ergosterol in their cellular composition, and causes a medical condition known as equine cutaneous pythiosis, which occurs in tropical and subtropical regions ([Gaastra et al., 2010](#), [Santos et al., 2011a](#), [Cardona et al., 2013](#)).

Equine is the species with the most reported cases, although there are also reports in canids (intestinal form), felines, wildlife, cattle, sheep ([Loreto et al., 2014](#) and humans ([He et al., 2016](#), [Chitasombat et al., 2020](#)). Skin lesions are more frequent on the distal parts of the limbs, ventral and lateral abdomen, pectoral region and mouth, perhaps because of the greater contact of these areas with potentially zoospore-contaminated water. Lesions are granulomatous ulcerative, with irregular borders, fistulous tracts or cavitations and with the presence of hard necrotic masses that detach easily ([Atiba et al., 2020](#), [Souto et al., 2021](#)).

Diagnosis is based primarily on observation of the clinical features of the skin lesions and histopathology ([Schanzembach et al., 2019](#)). Surgical resection of all affected tissue, in combination with an immunotherapeutic containing *P. insidiosum* antigens, is one of the most effective treatments currently available ([Mendoza et al., 2003](#); [Mendoza et al., 2004](#)).

This report describes the clinical characteristics of a case of pythiosis in an equine, with lesions focused in the palpebral region, its histopathological diagnosis and its treatment by immunotherapy.

CASE DESCRIPTION

An 11-year-old Quarter Horse mare was referred to the outpatient service of the Veterinary Hospital for Large Species of the Faculty of Veterinary Medicine and Zootechnics of the Veracruz University, for evaluation of a fast-growing granulomatous lesion that did not respond to treatment.

During the medical evaluation, it was observed that the mare presented a granulomatous ulcerative lesion with necrotic tissue and fibrin bloody exudate in the palpebral region of the left eye, measuring approximately 20 cm x 16 cm (Figure 1). During palpation of the affected area, the presence of fistulous tracts and yellowish-white caseified exudate was evident. The mare presented intense pruritus, pain in the affected region, pale mucous membranes and progressive weight loss. In the blood cytometry the relevant findings were anemia and leukocytosis due to neutrophilia.



Figure 1. Granulomatous ulcerative lesion with necrotic tissue and bloody fibrin exudate in the palpebral region of the left eye

Tissue samples were taken and preserved in 10% formalin for subsequent histopathological analysis. Histopathological evaluation showed an intense infiltrate of abundant eosinophils, reactive mast cells, presence of areas of multifocal necrosis and Splendore-Hoepli phenomenon (Figure 2); likewise, the presence of occasionally septate, branched and smooth-walled hyphae coinciding with *P. insidiosum* was identified (Figure 3).



Figure 2. Splendore-Hoepli phenomenon (hematoxylin and eosin staining, 40X)

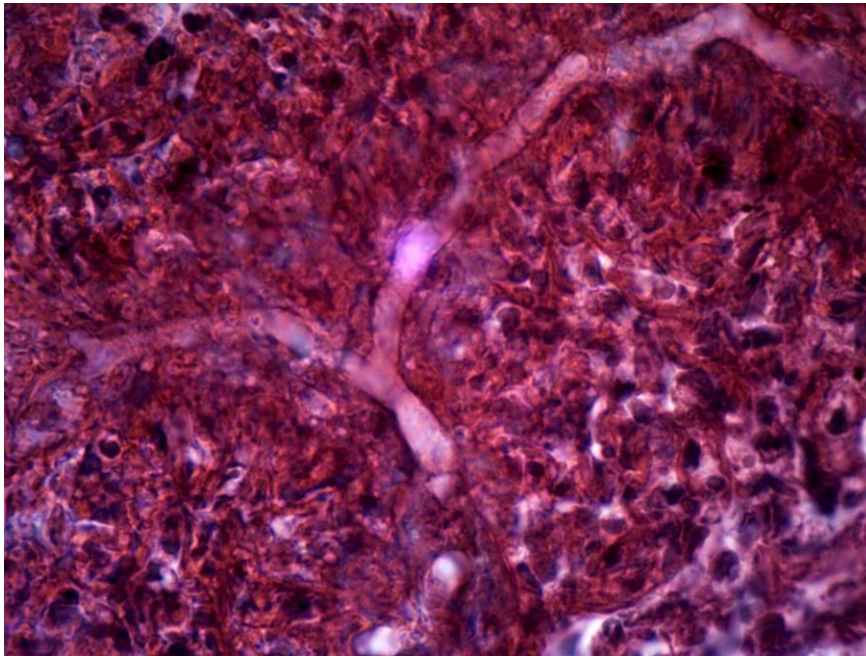


Figure 3. Branched and moderately septate hyphae of *Pythium insidiosum* (hematoxylin and eosin stain, 100X)

After clinical evaluation and histopathological findings, the affected tissue was removed by surgical resection with the mare on station. The mare was fasted for 12 hours prior to the surgical procedure, a 14 G catheter was placed in the jugular vein, and gentamicin (6.6 mg/kg IV), meglumine flunixin (1.1 mg/kg IV) and 22 000 IU/kg procaine penicillin were administered intramuscularly.

The mare was kept sedated with an initial bolus of 0.01 mg/kg detomidine hydrochloride and maintenance with a constant infusion of the same product, at a total dose of 10 mg in 250 mL of saline solution (0.04 mg/mL), and regional blocks were performed with 2% lidocaine hydrochloride. The affected area was prepared with aseptic technique with 8% povidone iodine and sterile water. During the surgical procedure it was possible to remove all the granulomatous tissue and concretions ("kunkers") and enucleation of the eyeball was avoided.

Due to the extent of the lesions and the risk of hyphae in deep tissues, it was decided to use a commercial immunotherapeutic developed from hyphal proteins and metabolites of *P. insidiosum*. The treatment consisted of applying 1 mL subcutaneously in the distal third of the neck on days 0 (day of surgery), 7 and 21 after the surgical procedure, according to the manufacturer's specifications (Pan American Veterinary Laboratories, Lexington, Texas®). After inoculation of the immunotherapeutic, no side effects were observed in the area of application.



Post-surgical treatment consisted of a compressive bandage, with a cross pattern, so as not to interfere with the field of vision of the contralateral eye and wound cleaning was carried out using isotonic solution every 24 hours. At the same time, she was maintained on gentamicin (6.6 mg/kg IV), procaine penicillin (22,000 IU/kg IM) and meglumine flunixin (1.1 mg/kg IV) for five days. During the postoperative period, the wound proliferation phase was monitored to ensure adequate granulation tissue formation and appropriate epithelialization and tissue remodeling.

DISCUSSION

Most of the cases reported in equines are in South America ([Salas et al., 201](#), [Cardona et al., 2014](#), [Dória et al., 2015](#), [Schanzembach et al., 2019](#), [Paz et al., 2021](#)) which is related to its tropical and subtropical regions. Veracruz state presents similar environmental conditions, for example, abundant rainfall that favors water stagnation in pastures and high temperatures, which benefit the etiological agent and its contact with equines ([Gaastra et al., 2010](#)).

The skin lesions found in the periorbital region of the mare agree with the description of granulomatous skin lesions described in other case reports in different countries ([Bezerra-Junior et al., 2010](#), [Mosbah et al., 2012](#), [Cardona et al., 2014](#), [Tartor et al., 2020](#)), where cutaneous lesions are reported in anatomical regions in direct contact with stagnant water contaminated with zoospores of *P. insidiosum*, so lesions in high regions of the head are not common. However, the first case with pythiosis lesions within the nasal cavity of a pregnant mare and antibodies for *P. insidiosum* in three horses was reported in Thailand ([Tonpitak et al., 2018](#), [Mar Htun et al., 2021](#)).

The diagnosis of pythiosis is primarily based on the observation of the clinical features of skin lesions. Similarly, it is important to establish the differential diagnoses of these lesions, as they are often misdiagnosed as cutaneous habronemiasis, exuberant granulation tissue, or sarcoids, so it is important to perform histopathological studies for definitive diagnosis.

The histopathological findings of this case agree with those described by [Márquez et al. \(2010\)](#), where the Splendore-Hoeppli phenomenon and the identification of septate hyphae could be observed. This phenomenon is considered an adaptive strategy of the etiological agent with the aim of ensuring its proliferation and survival in the host tissue, which can be confirmed by the presence of viable hyphae within the eosinophilic reaction ([Martins et al., 2012](#)).

The treatment of choice for *P. insidiosum* remains surgical resection of all affected tissue. However, some studies have shown that the combination of surgical resection and systemic antibiotics such as amphotericin B and miconazole, administered via regional



perfusion in the affected extremities, obtained a resolution of 92% (Worster *et al.*, 2000, Dória *et al.*, 2012). In contrast, Pires *et al.* (2013) only obtained 60% resolution of pythiosis skin lesions with amphotericin B. This type of combination therapy is performed when the lesion has invaded other types of anatomical structures, such as muscles, tendons and bones, which cannot be surgically removed.

In case surgical resection is partial due to the depth of the lesion, immunotherapy based on proteins from *P. insidiosum* hyphae can be used, which has had an efficacy of 90% in cases of acute lesions of less than 60 days and 20% in chronic cases that exceed two months (Mendoza *et al.*, 2003, Mendoza *et al.*, 2005). The immunological response was similar to that reported by Pereira *et al.* (2011), where seven days after inoculation of the first dose of the immunotherapeutic, the mare no longer showed evidence of *P. insidiosum* lesions or pruritus and began the wound remodeling phase (Figure 4).

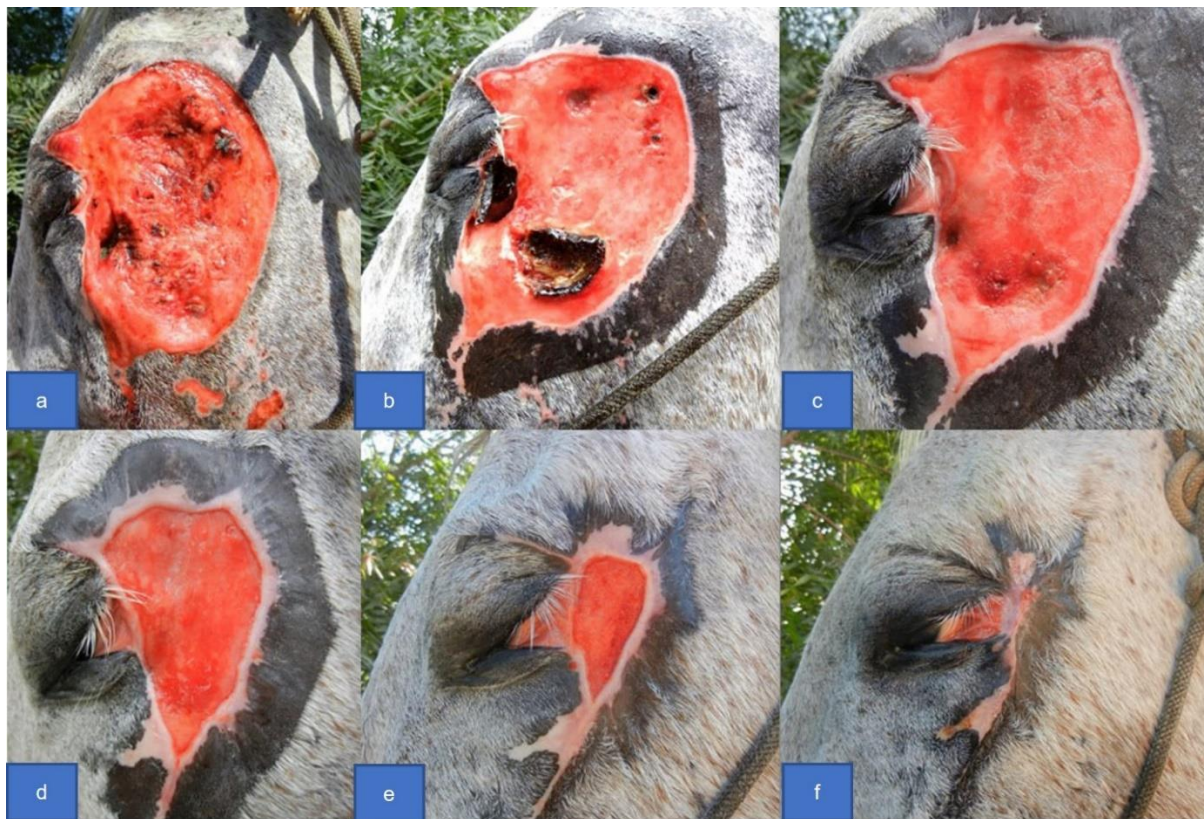


Figure 4. Initial phase, proliferation and remodeling. (a) Initial post-surgical lesion; (b-f) images show the proliferation and remodeling phases. At the end of the treatment only a scar lesion is seen with complete resolution of the case.



The success of immunotherapy is based on the fact that it promotes a shift from an antibody-mediated to a cell-mediated response. Normally, a type 2 (Th2) helper lymphocyte-regulated response predominates, with release of interleukins (IL) 4 and 5 and activation of eosinophils and mast cells, resulting in eosinophilic inflammation. In contrast, immunotherapy favors a response regulated by type 1 helper lymphocytes (Th1), with release of IL-2 and IFN- γ and activation of *T lymphocytes* and macrophages, which destroy *P. insidiosum* (Loreto *et al.*, 2014). Despite the curative properties of immunotherapy, Santos *et al.* (2011b) reported that the antibodies produced are probably not sufficient to prevent reinfection in the short and long term.

CONCLUSION

The successful outcome of treatments for cutaneous pythiosis depends critically on prompt diagnosis and early treatment with combination therapies for a good prognosis. This is the first case report documenting the diagnosis of *P. insidiosum* in equines in Veracruz State.

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