

Pythiosis rhinofacial zygomycosis in a hinny

Pitiose rinofacial em um muar

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Abstract

Equine pythiosis is a common disease in animals that graze in flooded areas of tropical and subtropical regions and it is caused by the oomycete *Pythium insidiosum*. The lesions are characterized by a granulomatous appearance of uneven surface and with fistulae, associated with fibrin-oozing bloody and caseous necrotic materials called *kunkers*, which are usually located in the abdomen or distal extremities. In this study, a case of pythiosis location of rhinofacial pythiosis on a hinny sent to the Pathology Laboratory at the Veterinary School Hospital (FURB) was here described. Necrotic foci surrounded by infiltrates of eosinophils, neutrophils, macrophages and plentiful fibrous connective tissue were observed in tissue samples from the region of nasal turbinates and face. Sparsely septate hyphae filaments were identified in the center of the necrotic areas. With the Grocott's methenamine silver staining, branched hyphae septate were also observed within the necrotic foci, thus confirming the diagnosis of rhinofacial pythiosis in a hinny.

Keywords: Facial. Hinny. Granuloma. Oomycete.

Resumo

A pitiose equina é uma doença comum em animais que pastoreiam em áreas inundáveis de regiões tropicais e subtropicais, sendo ocasionada pelo oomiceto *Pythium insidiosum*. As lesões são caracterizadas por sua aparência granulomatosa, de superfície irregular e com trajetos fistulosos, associada à exsudação



fibrino-sanguinolenta e material necrótico caseificado, denominado kunkers, que geralmente são localizadas no abdômen ou extremidades distais dos membros. No presente estudo, descreve-se um caso de pitiose de localização rinofacial em um muar enviado ao Laboratório de Patologia Veterinária do Hospital Escola Veterinário/FURB. Microscopicamente foram observados focos necróticos circundados por infiltrados de eosinófilos, neutrófilos, macrófagos e abundante tecido conjuntivo fibroso em amostras de tecidos da região de conchas nasais e face. Filamentos de hifas esparsamente septadas foram identificados no centro das áreas necróticas. Pela coloração de prata metenamina de Grocott foram observadas hifas septadas, ramificadas no interior dos focos de necrose, confirmando assim o diagnóstico de pitiose de localização rinofacial em um muar.

Palavras-chave: Face. Equídeos. Granuloma. Oomiceto.

Introduction

Pythiosis is a granulomatous disease, caused by anoomycete (*Pythium insidiosum*) which originates, more frequently, chronic injuries in horses. Moreover, it can also be observed in dogs, cattle, cats, birds, sheep, wild animals and humans (Gaastra et al., 2010). Some synonymies are used to describe Pythiosis such as Zygomycosis, Hifomicose, granuloma fibromycotic, Cancer of the Marshes "Swamp Cancer" as it is known in Florida, USA, or "Wound Brava" and "Wound of Fashion" as well as in Pantanal of Mato Grosso, place where its incidence reaches the highest levels in Brazil (Frey Jr. et al., 2007).

The disease has no predisposition by sex, age or breed and it can involve tissues such as bones, organs of the digestive system, lymph nodes, eyes, arteries (Frey Jr et al., 2007) and nasal mucosa (D'Ultra Vaz et al., 2009). The etiologic agent *Pythium insidiosum* is not classified as a fungus, being therefore in accordance with the phylogenetic analysis, most related to algae and ditomaceous (Gaastra et al., 2010).

The source of infection is environmental zoospores and there are no reports of direct transmission neither between animals nor between animals and human beings. It is an aquatic microorganism, which is characterized by the formation of biflagellate zoospores, coming from filamentous sporangia that are the way wherein the agent spreads. These microorganisms are released periodically in swampy waters with aquatic vegetation and temperature between 30 and 40 °C (Miller and Campbell, 1982), and infects horses and other mammals that go into these places. Mobile zoospores are attracted

to animals' hair and penetrate their skin through preexisting lesions, producing the disease (Sallis et al., 2003).

The clinical course of the disease varies from days to months depending on the time course of skin lesions, which are characterized by association with ulcerative abundant granulation tissue and serum-bloody exudation. Masses of necrotic tissue with a white-yellowish color can be observed in its interior. These masses are called *kunkers* (Frey Jr. et al., 2007). Injuries are usually located in the belly or distal extremities, once these sites are in close contact with moist environment for the animals enter rivers and marshy areas (Luvizari et al., 2002).

These lesions must be differentiated from cutaneous habronemiasis caused by deposition of spirudis larval (Sallis et al., 2003). Other injuries should be considered in the differential diagnosis including equine sarcoid and skin granulomas as those caused by *Conidiobolus spp* and *Basidiobolus haptosporus* (Sallis et al., 2003). The diagnosis is based on clinical signs, histopathological findings, antibody detection, isolation and identification of the agent (Gaastra et al., 2010). Other methods for laboratory diagnosis, immunohistochemistry (Pedroso et al., 2009, Gaastra et al., 2010), immunodiffusion (Miller and Campbell, 1982), enzyme-linked immunosorbent assay - ELISA (Leal et al., 2001) and molecular methods (Azevedo et al., 2012) have also been used. Santurio et al. (2006) used an indirect ELISA test for the diagnosis of Pythiosis in horses, which allowed a sensitivity observation of 97.72% and a specificity of 90.27%.

This study was intended to report a case of facial pythiosis with involvement of nasal turbinates on a hinny in the state of Santa Catarina.

Case Report

A male hinny, about 16 years old, used for working and riding, presented an extensive and hard to heal wound on the facial region. The injury rapidly and progressively grew being refractory to treatment with antibiotics, anti-inflammatory, antiparasitic and potassium iodide, the last one used orally and topically. After four months of its inception, it expanded reaching all the right face from the lacrimal bone region until nostrils, surpassing the midline of the skull and invading the left cheek. Its lesional aspect showed granulomatous inflammation, ulcerated in places, with edematous and irregular edges, covered by serosanguinous exudate and fetid odor. The animal presented inspiratory dyspnea, which indicated that the nasal cavity was also

affected. As irritability was great, the animal did not properly feed itself and consequently lost weight. In the property, the hinny was raised extensively with other animals having access to a swampy area with a small water course which formed a swampy place. The animal had also close contact with a mare, which had no consistent signs with the disease. On the clinical examination, the animal showed physiological parameters within the normal range for the species. Because of the extent of injury and bodily weakness, the animal was euthanized. At necropsy it was found that the skin lesion was granulomatous containing fistulae, filled with necrotic yellow, dry and crumbly (*kunkers*) material that easily detached from the surrounding fibrovascular tissue. Through a median section of the head, the same type of injury on the first third of the dorsal and ventral turbinate could be observed (Figure 1). Despite the undermining in both turbinate, no gross injuries were observed in other regions of the respiratory tract. Tissue fragments were collected and fixed in 10% formalin solution for 48 hours and routinely processed in the Pathology



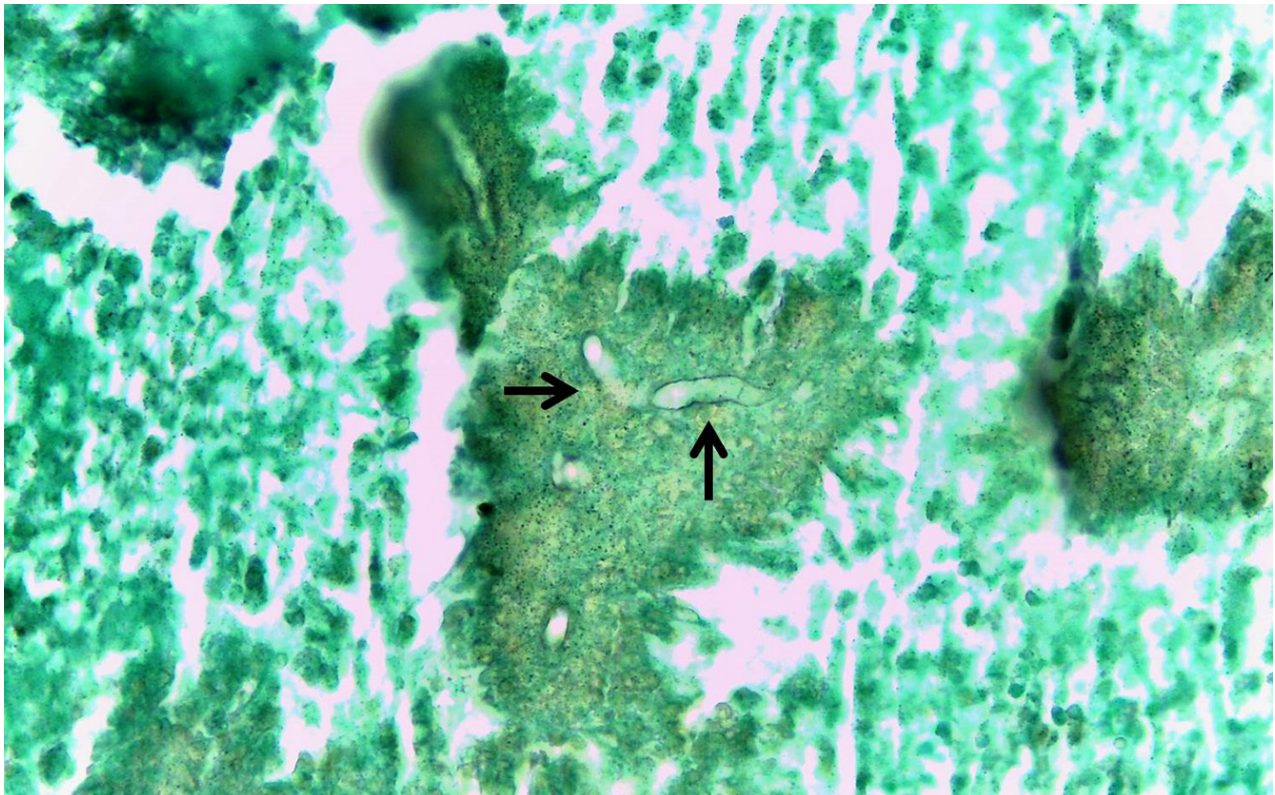
Note: (A) Face, skin granulomatous inflammation, ulcerated in places, with edematous and irregular edges, covered by serosanguinous exudate. (B) First third of the dorsal and ventral turbinate, granulomatous lesion containing necrosis, dry and crumbly (*kunkers*).

Figure 1 - *Ptidium insidiosum*, skin lesion and nasal turbinate.

Laboratory, at the Veterinary School Hospital (FURB). Histology slides with hematoxylin and eosin (HE) were prepared and immunohistochemical Grocott's methenamine silver (GMS) technique was performed.

Histological evaluation of skin injuries and nasal turbinate showed multiple granulomas and pyogranulomas with central neutrophils and

eosinophils besides a considerable amount of macrophages and some peripheral multinucleated giant cells. There were some unstained hyphae in transversal and longitudinal sections (Figure 2). Occasionally, these sections were surrounded by radiating eosinophilic materials, morphologically consistent with the *Splendori Hoeppli* reaction.



Note: Photomicrograph showed some hyphae transversal and longitudinal sections (black arrow). GROCCOTT, obj. 40x.

Figure 2 - *Ptidium insidiosum*, skin and nasal turbinate.

Through Grocott's methenamine silver (GMS) technique, the incidence of branched hyphae was possible to be observed, sometimes chambered in the center of the granulomas.

Discussion

An exuberant, irregular proliferative skin lesion was described in the present case, with exudative character. Gaastra et al. (2010) described the presence

of *kunkers*, marked fibrosis and neovascularization, features that are common to cutaneous pythiosis in horses. Through Grocott immunohistochemical method, small chambered structures, similar to hyphae, displayed at the necrotic center of the injuries, showed markers blackened in the outline, screening thus to be compatible with *Pythium insidiosum*. In Santa Catarina, Brazil, the scientific reports of pythiosis are scarce, regardless the species. The lack of regional epidemiological data implies a deficit of information, which directly influences the diagnostic and prophylactic disease in that state.

There are several reports of pythiosis in horses in different regions of Brazil, as in the states of Mato Grosso and Mato Grosso do Sul (Leal et al., 2001), Paraíba (Tabosa et al., 1999), Paraná (Headley and Arruda Jr., 2004), Rio de Janeiro (Sanavria et al., 2000), Rio Grande do Sul (Santos et al., 1987), São Paulo (Rodrigues and Luvizoto, 2000), Sergipe (Bandeira et al., 2009), and Minas Gerais (Maciel et al., 2008). Reports of pythiosis in calves were registered in the region of Pantanal in Mato Grosso, Brazil (Santurio et al., 1998).

Based on the information provided by the weather information service (Climatepro, 2014) upon the investigated area, it was found that the analyzed case of Pythiosis occurred in the very beginning of the warmest and rainiest season, a period where the average rainfall was 169 mm and the average temperature was 24 °C. Similar data to those seen in other regions (Leal et al., 2001), demonstrating that climatic conditions favor the growth of the microorganism in the environment. The proliferation of the zoospores required temperature between 30 and 40 °C and accumulation of water in wetlands (Miller and Campbell, 1982).

Although the average temperature is lower than the reported in literature it is known that in standing water the temperature varies with several factors, such as soil depth, solar radiation, cloud cover, time of day and the presence of winds. Moreover, water has the ability to retain heat, which contributes to thermal fluctuations (Angelocci and Villa Nova, 1995). Another important factor for the development of injuries is related to the component that the affected animals remain for long periods in contact with stagnant water (Chaffin et al., 1995).

There are few reports of facial pythiosis in horses (Bandeira et al., 2009, D'Ultra Vaz et al., 2009). The most common, report injuries to limbs and abdomen because these areas remain in contact with wetlands for a longer period (Sallis et al., 2003; Headley and Arruda Jr., 2004; Santurio et al., 2006).

Considering all the depicted aspects, it is possible to be concluded that the clinical, lesional and epidemiological findings of this report are unusual. There are few descriptions of pythiosis in hinny species in literature, especially with this type of lesion distribution. In the State of Santa Catarina, Brazil, climate and environmental conditions favor the

occurrence of pythiosis. However, data concerning this pathology is still scarce, reinforcing the necessity of further studies about the topic.

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