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Original Article
Livestock Diseases

Cutaneous pythiosis in equines in the Amazon Biome[1]

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ABSTRACT.- Barbosa J.D., Oliveira H.G.S., Bosco S.M.G., Silveira N.S.S., Barbosa C.C., Brito M.F., Oliveira C.M.C. & Salvarani F.M. 2023. **Cutaneous pythiosis in equines in the Amazon Biome.** *Pesquisa Veterinária Brasileira 43:e07167, 2023*. Instituto de Medicina Veterinária, Universidade Federal do Pará, BR-316 Km 61, Saudade II, Cristo Redentor, Castanhal, PA 68740-970, Brazil. E-mail: felipems@ufpa.br

The study aimed to describe the clinicopathological aspects of 37 cases of pythiosis, 34 in horses and three in mules, from properties located in the Amazon biome of Pará, Brazil. The clinical signs observed in the animals were weakness, poor-to-regular nutritional status, pale mucous membranes, itching at the lesion site, and lameness when the limbs were affected. The lesions were located on the lips, nostrils, rib region, thoracic and abdominal walls, scapular, distal limbs, foreskin, perineum and udder. Macroscopically, ulcerative and granulation-tissue-like masses were observed, with fistulous tracts filled with yellowish and foul-smelling serosanguinous discharges. In the biopsy, it was possible to visualize white and firm areas with foci of yellowish necrotic material and hardened masses, called kunkers. The histopathological examination showed a pyogranulomatous inflammatory reaction with the presence of *Pythium insidiosum* hyphae, which were impregnated with black, confirming the diagnosis of pythiosis in equids in the Amazon biome, being the first report of the disease in mules in the region.

INDEX TERMS: *Pythium insidiosum*, mules, diagnosis, cutaneous mycosis, kunkers.

RESUMO.- [**Pitiose cutânea em equídeos no Bioma Amazônico**.] O trabalho objetivou descrever os aspectos clínico-patológicos de 37 casos suspeitos de pitiose, 34 em equinos e três em muares, provenientes de propriedades localizadas no Pará, bioma amazônico brasileiro. Os sinais clínicos observados nos animais eram caracterizados por debilidade, estado nutricional de ruim a regular, mucosas pálidas, prurido no local da lesão, além de claudicação quando os membros foram acometidos. As lesões eram localizadas nos lábios, narinas, região das costelas, parede torácica e abdominal, escapular, distais dos membros, prepúcio, períneo e úbere. Macroscopicamente observavam-se extensas lesões ulceradas com intensa proliferação de tecido de granulação, de bordos irregulares, com tratos fistulosos, de consistência firme, denominados de *"kunkers"*, preenchidos com material amarelado e friável, possuindo exsudação serossanguinolenta de odor fétido. Ao exame histopatológico observou-se reação inflamatória piogranulomatosa com presença de hifas de *Pythium insidiosum,* as quais se impregnaram de negro, confirmando o diagnóstico de pitiose em equídeos no Bioma Amazônico, sendo o primeiro relato da doença em muares na região.

TERMOS DE INDEXAÇÃO: *Pythium insidiosum,* muares, diagnóstico, micose cutânea, *kunkers*.

INTRODUCTION

Pythiosis is a cutaneous, gastrointestinal or multisystemic pyogranulomatous disease that affects equines, canines, bovines, ovines, caprines, felines, birds, wild animals and humans (Gaastra et al. 2010, Carmo et al. 2015, Ribeiro et al. 2017, Vilela et al. 2018, Bezerra et al. 2020, Souto et al. 2022). *Pythium insidiosum* is an oomycete whose biological cycle is characterized by the formation of infective, motile

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and biflagellate aquatic zoospores. When released into the aquatic environment, they undergo chemotaxis by animal hairs and tissues and penetrate the skin through a break in continuity or through the follicular ostium in cases where there are no previous lesions, potentially leading to chronic disease (Mendoza et al. 1993, Santurio et al. 1998, Fonseca et al. 2014).

The occurrence of pythiosis in animals is increasing worldwide, while human cases of pythiosis are more frequent in Thailand (Krajaejun 2014); however, in Brazil, cases have also been reported in humans (Bosco et al. 2005), which may present as ocular or vascular forms. In equines, the most common clinical presentation is cutaneous, nasal and intestinal (Leal et al. 2001, Galiza et al. 2014). The risk factor for humans and animals is contact with flooded areas (Gaastra et al. 2010, Krajaejun 2014). Several therapeutic strategies and drugs have been studied (Santos et al. 2011a, Zanette et al. 2013), but conventional antifungal drugs are not effective against the agent (Krajaejun 2014). Information about the biological cycle of *P. insidiosum* is limited, and the diagnosis and control of the disease are difficult due to the epidemiological characteristics of particular regions, as in the case of the Amazon Biome and the Pantanal (Fonseca et al. 2014). Successful treatment is related to the time of evolution, location and size of the lesion, age and physiological state of the animal (Santos et al. 2011a, 2011b).

In Brazil, this disease causes losses in the equine industry due to expenses with treatment, unusable animals and death (Santos et al. 1987, Sallis et al. 2003, Pierezan et al. 2009, Martins et al. 2012, Galiza et al. 2014). Pythiosis in equines affects all regions of Brazil (Leal et al. 2001, Santos et al. 2011a, 2011b, Marcolongo-Pereira et al. 2012, Pessoa et al. 2014, Reis-Gomes et al. 2018, Viana et al. 2020, Souto et al. 2021). However, in the Amazon, which presents different epidemiological factors for the occurrence of the disease, such as high rainfall rates, high temperatures, high relative humidity, and large flooded areas, which favor the maintenance of oomycetes in the environment, there are still few studies on the frequency of the disease in the Amazon biome. Therefore, due to the scarcity of research and the importance of pythiosis in the Amazon region, the present study aimed to describe the clinicopathological aspects of horses affected by *P. insidiosum* in Pará, Brazil.

MATERIALS AND METHODS

Technical visits were made to 21 rural properties in Pará, Amazon, Brazil, where 37 animals were treated: 34 horses and three mules with clinical signs of pythiosis. The clinical histories of the properties were obtained from owners, and the clinical examination of all animals was carried out, with collection of biological samples for histopathological and microbiological diagnosis. The tissue fragments collected during the biopsies were fixed in 10% formalin and sent to the Pathology Sector of the Universidade Federal Rural of Rio de Janeiro (UFRRJ), which were routinely processed for histopathology and subjected to Grocott's silver nitrate methanamine technique (Suvarna et al. 2013). The kunkers also collected at the biopsies were refrigerated and sent to the Mycology Laboratory of the "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (Unesp), Botucatu campus, to be cultured and isolated, according to Grooters et al. (2002).

RESULTS

Thirty-four horses (identified as 1-34) were from 20 different rural properties located in the municipalities of Aurora do Pará, Capanema, Castanhal, Irituia, Santa Maria do Pará and Xinguara, and the three mules (identified as 35-37) were from a farm in the municipality of Eldorado dos Carajás, all in the State of Pará. The equids were of different breeds, with ages between one and 10 years old, of both sexes and raised in an extensive system. According to the history, in the hours of higher temperatures of the day, the animals remained for long periods in flooded areas (Fig.1). In the beginning of the disease, horses and mules presented good nutritional status; however, with the evolution of the clinical signs, there was loss of body condition; they became weak, and their mucous membranes were pale. Pruritus in the injured area was frequent and intensified with the evolution of the disease; some animals scratched their wounds excessively with their mouths (Fig.2), mutilating themselves and sometimes causing profuse bleeding. Serosanguinolent exudate flowed from the wounds (Fig.3). Lameness was observed in animals with lesions located on the limbs.

Seventeen horses (17/34) showed lesions on the limbs, nine with lesions in the ventral region of the abdomen (Fig.4), one with lesions on the chest, one with lesions on the thorax and one with lesions on the prepuce. In one of these horses (Equine 14), there were lesions in two areas, the medial region of the thigh and the tail (Fig.5). Four animals showed lesions in the distal regions of the limbs, which affected the hoof coronet, heels and gait, leading to partial loss of the horny case (Fig.6). Lesions on the dorsal regions were observed in five horses on the lower lip (Fig.7), nose and scapular region with one case each, and two horses had lesions on the perineum. Of the three mules, two presented lesions in the distal region of the hind limbs, and one female presented lesions in the udder region.

The skin lesions macroscopically showed extensive ulcerative and granulation-tissue-like masses, ranging from 3 to 50cm in diameter, with fistulous tracts and a firm consistency; these lesions, which are called kunkers (Fig.8), were filled with a yellowish and foul-smelling serosanguinous discharge. All the horses exhibited kunkers (Fig.9) of different sizes, and the lesions were restricted to the areas of the body that were in contact with water.

Histopathology showed multiple negative images of hyphae that in some cases were associated with amorphous eosinophilic material and in other areas with a granular pattern similar to eosinophil granules (Fig.10). A pyogranulomatous inflammatory reaction involving eosinophils, macrophages, lymphocytes, plasma cells, marked fibroplasia and vascular endothelial proliferation was also observed. Microbiological diagnosis showed large and branched cenocytic hyphae. Grocott's staining revealed black hyphae with parallel walls and branched and sparsely septate that corresponded to *P. insidiosum* (Fig.11). In culture and isolation on Sabouraud agar at 27°C for seven days, *P. insidiosum* was identified, and in the first 24 hours of incubation, it was already possible to observe the appearance of the filamentous colony, hyaline and membranous texture, characteristic of the agent (Fig.12-13).

DISCUSSION

Pythiosis is popularly known in the Amazon region as a "sponge", with habronemiasis as the main differential diagnosis; in general, it is difficult to differentiate these diseases in the field without the aid of laboratory tools. In cutaneous habronemiasis, areas of necrosis might be mistaken for kunkers; however, in these areas, there are no cylindrical structures inside fistulous tracts. Other differential diagnoses include exuberant granulation tissue, sarcoid, squamous cell carcinoma and melanoma (Leal et al. 2001, Grooters et al. 2002, Santos et al. 2011a, 2011b, 2014, Martins et al. 2012, Galiza et al. 2014, Pessoa et al. 2014).

As in other reports in Brazil, in the Amazon epidemiologically, horses with pythiosis show no predisposition to breed, age or sex (Leal et al. 2001, Martins et al. 2012, Galiza et al. 2014, Pessoa et al. 2014). The predisposing epidemiological factors for the occurrence of the disease in the Amazon biome are the favorable environmental conditions of temperature and humidity (Santos et al. 2011a, 2011b, Marcolongo-Pereira et al. 2012), which in the state of Pará range between 23 and 32°C and 60 to 91%, respectively (INMET 2022).

The lesions were predominantly in the ventral abdominal region and limbs and occurred in a single form. In only one case (Equine 14), there were lesions in two distinct regions (left hind limb and tail). The localization of the lesions may be associated with the habits observed in all properties of the affected animals, such as staying for long periods in flooded areas in an attempt to alleviate heat or feeding on aquatic plants, favoring the long contact of the skin with water; this creates a favorable environment for development of the disease (Leal et al. 2001, Martins et al. 2012, Galiza et al. 2014, Pessoa et al. 2014).

Fig.1-4. Pythiosis in horses in the Amazon region. (**1**) Animal with a large part of its body submerged in water in a marshy area of the Amazon region; favorable ecological conditions for the development of pythiosis. (**2**) Animal with pythiosis in the right hind limb and in a self-mutilation posture due to itching. (**3**) Ulcerated lesion with irregular borders, granulation-tissue-like masses with serosanguinous discharges, on the right ventrolateral region. (**4**) Animal with pythiosis in the ventrolateral region of the abdomen. The lesion had irregular areas of ulcerative and granulation-tissue-like masses with serosanguinous discharges.

In this study, the first in the indexed scientific literature, kunkers were observed in all horses with suspected pythiosis, whose width ranged from 0.5 to 4cm, which in some cases occupied extensive areas in the lesions. Kunkers of different sizes were also described in pythiosis of other animal species, as by Videla et al. (2012), who reported kunkers of 5 to 10mm in the vulvar region of two camelids (*Camelus dromedarius*), and Ubiali et al. (2013), who reported the presence of kunkers of 1 to 5mm in the mucocutaneous region of the nostrils of two sheep among 14 sheep affected by the rhinofacial form of pythiosis.

The morphology of hyphae compatible with *P. insidiosum* and the pyogranulomatous inflammation composed of microscopic

Fig.5-8. Pythiosis in horses in the Amazon region. (**5**) Animal with pythiosis on the tail; this animal also had a lesion on the medial aspect of the thigh. (**6**) Ulcerated and proliferative lesion in the distal region of the left hind limb. There is involvement of the pastern and invasion into the coronet and heel regions and partial loss of the horny case. (**7**) Animal with pythiosis in the mouth, with a severe ulcerated and proliferative lesion on the lower lip. (**8**) Foci of yellowish necrotic tissue, the kunkers, surrounded by fibrous connective tissue areas of the animal in Figure 6.

findings are highly suggestive of pythiosis (Sallis et al. 2003, Martins et al. 2012, Fonseca et al. 2014). In this study, the histological lesions were compatible with those reported for pythiosis in the literature, and the final diagnosis was based on the dataset from the macroscopic evaluation of the lesions, histopathology and isolation of the agent. Due to the difficulty of culturing the agent, the morphological diagnosis should be enhanced by biopsy and histological analysis. The fragment of the lesion to be sent for histopathological examination should contain kunkers, avoiding the collection of lesions composed only of granulation tissue. For microbiological exams, kunkers are the most indicated for culture in selective media (Grooters et al. 2002). For molecular analyses, fresh or frozen kunkers should be sent (Azevedo et al. 2012).

There was the identification of the occurrence of pythiosis in the udder of a mule, this being the first report described in the literature. Ribeiro et al. (2022) reported the presence of two granulomatous lesions with a diameter of 30 cm near the udder of a pregnant mare, and Souto et al. (2019) identified mastitis caused by *P. insidiosum* in mares, among which 142 cases of cutaneous pythiosis were identified in three lesions that extended to the mammary glands. Macroscopically, the mammary gland was edematous, with multifocal areas of ulceration measuring from 1 to 3.5cm, and when cut, they presented fistulous tracts with the presence of serosanguinous secretion, as observed in this case. The diagnosis was confirmed by epidemiological, clinical, anatomopathological and immunohistochemical findings. The authors consider mastitis a relatively uncommon consequence of cutaneous pythiosis in horses but reinforce that it should be included as a differential diagnosis of mastitis.

This study seems to be the first to present the clinical, epidemiological, histological and microbiological diagnosis of pythiosis in equines (horses and mules) in the Amazon biome, confirming that this is an important cutaneous disease and that the ecological conditions of the Amazon region are highly favorable to the emergence of the disease. Due to previous diagnoses of pythiosis in humans in Brazil (Bosco et al. 2005, Fonseca et al. 2014), it is important to alert medical professionals to the possible risk of pythiosis in humans in the Amazon region and the need for further studies evaluating the disease from the perspectives of public health or the equine sector, an important economic arena in northern Brazil.

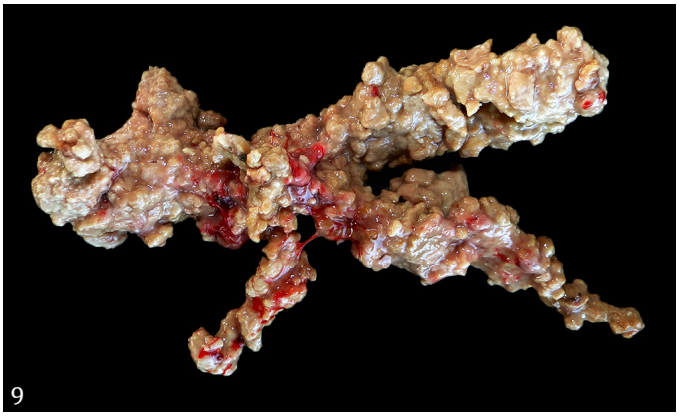

Fig.9. Pythiosis in horses in the Amazon region. Kunker dissected from the lesion of pythiosis in the ventral region of a horse.

CONCLUSION

Pythiosis occurs in Pará; however, more studies and diagnostics should be carried out to elucidate the real importance of this disease in the state and in the Amazon biome. It is a disease that deserves to be highlighted due to its rapid evolution and difficulty of diagnosis that results in treatment failures, leading to high economic losses in equine breeding, especially in northern Brazil. The epidemiological data, as well as the clinical signs and macroscopic characteristics of the lesions associated with histopathological and microbiological examination, are of fundamental importance to ensure the correct and early diagnosis of this disease. This may be the first report of pythiosis in the udder of a mule described in the scientific literature.

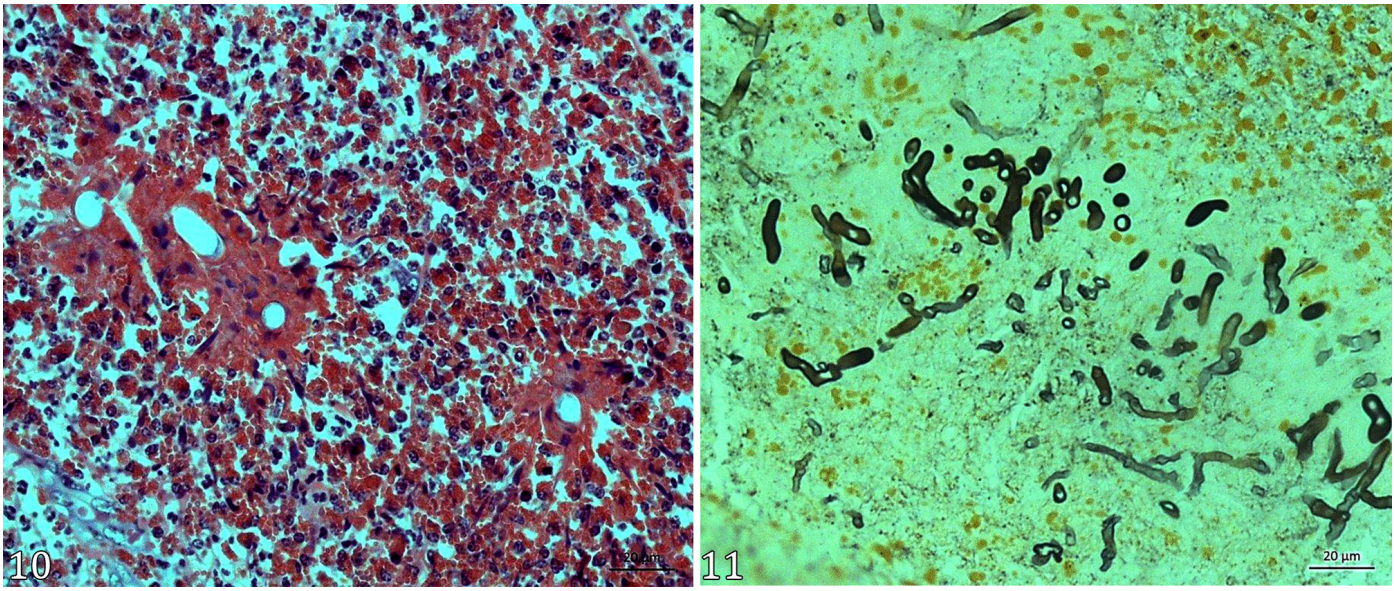

Fig.10-11. Pythiosis in horses in the Amazon region. (**10**) Negative images of hyphae surrounded by a large number of eosinophils. HE, obj.63x. (**11**) Cenocytic, branched, and rarely septate hyphae of *Pythium insidiosum*. Grocott, obj.63x.

Fig.12-13. Pythiosis in horses in the Amazon region. Macroscopic aspect of the filamentous, hyaline, velvety and membranous colony. Microscopic aspect of *Pythium insidiosum* cultures grown on Sabouraud agar at 27°C for 7 days. Presence of cenocytic, broad, branched hyphae. Lactophenol cotton blue, obj.20x.

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