



Rhinofacial pythiosis with pulmonary and lymphatic dissemination in a Uruguayan horse

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ABSTRACT: We described the first case of rhinofacial pythiosis with dissemination in an adult horse in Uruguay. The affected horse presented a partially circumscribed mass measuring 30 x 23 x 9 cm affecting the right side of the face with involvement of cutaneous, subcutaneous, and muscular tissues, the right nasal cavity, and adjacent structures. At *postmortem* examination, the main lesions were “kunkers” characterized by pyogranulomatous and eosinophilic dermatitis, Splendore-Hoeppli reaction and numerous intralésionales hyphae immunolabeling with polyclonal anti-*Pythium insidiosum* antibody. Similar lesions were observed in the lung and submandibular lymph nodes. The diagnosis was made by the characteristic histological lesions and the strong immunolabelling by anti-*P. insidiosum* antibody.

Key words: Equine, *Pythium insidiosum*, Tacuarembó river.

Pitiose rinofacial com disseminação pulmonar e linfática em um equino no Uruguai

RESUMO: Descreve-se o primeiro caso de pitiose rinofacial com disseminação em um equino adulto no Uruguai. O animal apresentava uma massa parcialmente circunscrita medindo 30 x 23 x 9 cm acometendo o lado direito da face e com envolvimento dos tecidos cutâneo, subcutâneo e muscular, cavidade nasal direita e estruturas adjacentes. Os principais achados patológicos incluem a formação de kunkers microscopicamente caracterizados por dermatite piogranulomatosa e eosinofílica, reação de Splendore-Hoeppli, e numerosas hifas intralésionais imunomarcadas com anticorpo policlonal anti-*Pythium insidiosum*. Lesões semelhantes foram observadas no pulmão e nos linfonodos submandibulares. O diagnóstico foi baseado nas lesões histológicas características e imunomarcagem com anticorpo anti-*P. insidiosum*.

Palavras-chave: Equinos, *Pythium insidiosum*, Rio Tacuarembó.

INTRODUCTION

Pythium insidiosum is an oomycete that affects several animal species worldwide, with multiple clinical manifestations (WHITE et al., 2008; GAASTRA et al., 2010; SANTOS et al., 2014; ROMERO et al., 2019; TARTOR et al., 2020; CARMO et al., 2021; SOUTO et al., 2021; SOUTO et al., 2022). Equine pythiosis is characterized mainly by the cutaneous/subcutaneous presentation (SOUTO et al., 2022), affecting the lower limbs, thorax, or ventral abdominal walls (GAASTRA et al., 2010).

Rhinofacial pythiosis is rarely described in horses (REIS JUNIOR et al., 2003; SOUTO et al., 2016; TONPITAK et al., 2018; ROMERO et al., 2019); although, this clinical presentation has

frequently been observed in sheep in which the nasal cavity is one of the most affected anatomical sites (RIET-CORREA et al., 2008; SANTURIO et al., 2008; PORTELA et al., 2010; UBIALI et al., 2013; CARMO et al., 2021; SOUTO et al., 2022; FIRMINO et al., 2023). In horses, the spread of pythiosis to other organs from an initial injury is also rarely reported (REIS JUNIOR et al., 2003; SOUTO et al., 2016). The pathogenic mechanism of disseminated pythiosis is elusive (REIS JUNIOR et al., 2003). In this report, we described the clinicopathological findings of a case of equine rhinofacial pythiosis with dissemination to the lung and regional lymph nodes.

A 14-year-old Criollo mare was presented with a history of a two-year progressive weight loss, dyspnea, and marked unilateral facial swelling with

purulent discharge, which had been previously treated with penicillin (250 mg/kg, q 24 h for 3 days). The case occurred on a farm located in the Department of Tacuarembó, where the animal was kept in a pasture with many areas of stagnant water. The farmer only sought veterinarian assistance when the progressive swelling led to difficulties in eating. The facial swelling caused a deviation of the nasal cavity and was characterized by an irregular, infiltrative, and ulcerated mass with serosanguineous discharge. The unfavorable prognosis led to euthanasia and posterior *postmortem* examination.

The rhinofacial lesion had a tumoral-like appearance characterized by a partially circumscribed mass measuring 30 x 23 x 9 cm affecting the right side of the face (Figure 1A) with the involvement of cutaneous, subcutaneous, and muscular tissues, as well as an extensive lesion in the right nasal cavity and adjacent structures (maxillary bone and cartilages). The horse also exhibited facial asymmetry. A thorough examination revealed fistulas with a mucopurulent to

serosanguineous discharge and small yellow hard necrotic masses at the cut surface (kunkers) within fistulous cavitations. The submandibular lymph nodes were markedly increased. In addition, the lungs exhibited a well-delimited, firm nodular lesion of 3 cm in diameter in the left lobe with kunkers in the cut surface (Figure 1B).

Samples of the rhinofacial lesion, submandibular lymph nodes, and lung were fixed in 10% neutral buffered formalin, processed routinely for histopathology, and stained with hematoxylin and eosin and Grocott's methenamine silver stain. Immunohistochemistry was conducted using a polyclonal anti-*P. insidiosum* antibody, according to MARTINS et al. (2012) in samples of the nasal cavity and cutaneous lesions.

Histopathological examination of the rhinofacial lesion revealed a marked inflammatory infiltrate predominantly composed of eosinophils and neutrophils along with macrophages, plasma cells, and multinucleated giant cells within the mucosal,

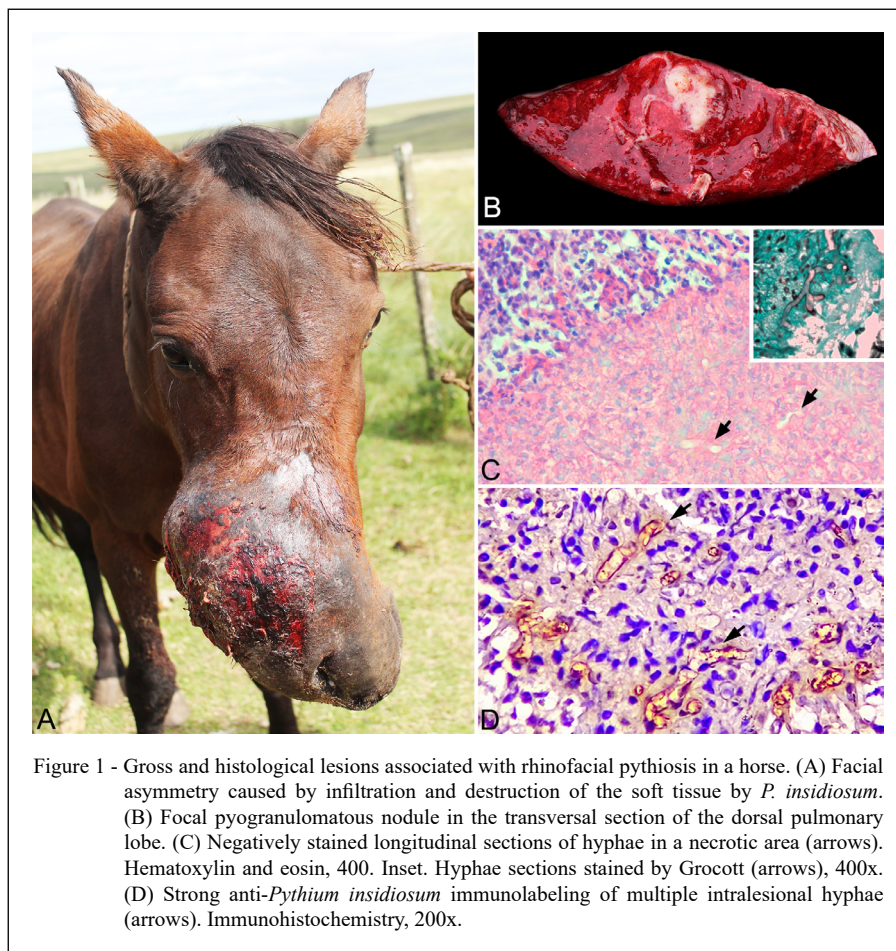


Figure 1 - Gross and histological lesions associated with rhinofacial pythiosis in a horse. (A) Facial asymmetry caused by infiltration and destruction of the soft tissue by *P. insidiosum*. (B) Focal pyogranulomatous nodule in the transversal section of the dorsal pulmonary lobe. (C) Negatively stained longitudinal sections of hyphae in a necrotic area (arrows). Hematoxylin and eosin, 400. Inset. Hyphae sections stained by Grocott (arrows), 400x. (D) Strong anti-*Pythium insidiosum* immunolabeling of multiple intralesional hyphae (arrows). Immunohistochemistry, 200x.

cutaneous, subcutaneous, and muscular tissue, with multifocal to coalescent areas of necrosis and collagenolysis surrounded by abundant fibroplasia and granulation tissue. The necrotic areas were eosinophilic with negatively stained longitudinal and transverse sections of hyphae (Figure 1C). Those structures could only be found within these eosinophilic necrotic areas frequently associated with Splendore-Hoeppli reaction. The lung and lymph nodes presented the same eosinophilic granulomatous inflammation observed in the rhinofacial region. Cutaneous, subcutaneous and lung sections stained by Grocott's methenamine silver stain revealed 2-7 μm tubular structures with parallel walls (Figure 1C). Immunohistochemistry performed in cutaneous sections from the rhinofacial lesion revealed strong immunolabeling of these structures by polyclonal anti-*P. insidiosum* antibody (Figure 1D).

We present an unusual case of equine rhinofacial pythiosis with secondary involvement of lungs and lymph nodes. The diagnosis was based on anatomopathological findings and confirmed by immunohistochemistry. Rhinofacial pythiosis frequently affects sheep (RIET-CORREA et al., 2008; SANTURIO et al., 2008; PORTELA et al., 2010; CARRERA et al., 2013; UBIALI et al., 2013; BERNARDO et al., 2015; MUSTAFA et al., 2015; CARMO et al., 2021; SOUTO et al., 2022; FIRMINO et al., 2023) and has only been reported in a few horses in previous studies (REIS JUNIOR et al., 2003; SOUTO et al., 2016; ROMERO et al., 2019).

The route of infection for the oomycetes in similar cases with rhinofacial clinical manifestations is attributed to direct contact with contaminated water during drinking (SOUTO et al., 2016), with the involvement of a previous lesion in the oral cavity that would extend to the nasal cavity (CARRERA et al., 2013; BERNARDO et al., 2015). Although, a complete examination could not reveal any lesion on the oral cavity, the destructive gross appearance of the mass may have hampered the identification of a previous lesion. The epidemiological dynamics of pythiosis in horses are complex and not fully understood, and more aspects may be involved in this case. A recent study correlated pythiosis lesions in horses with the warmer areas of the animal body, predominating in dark-coated anatomical sites and animals such as this horse. Those aspects together with the high atmospheric temperatures and rainy period of occurrence could add to the hypothesis stated by the study of a possible implication of hematogenous vectors in the transmission of the disease (SANTOS et al., 2024). However, the data available is not enough to

verify that hypothesis, and further research should be conducted to better understand all the epidemiological aspects that could be involved in the infection.

Disseminated pythiosis in sheep with the nasal form of the disease has been described with the involvement of different organs such as lymph nodes, lungs, central nervous system, and parotid glands (RIET-CORREA et al., 2008; SANTURIO et al., 2008; PORTELA et al., 2010; CARRERA et al., 2013; UBIALI et al., 2013; MUSTAFA et al., 2015; SOUTO et al., 2016; FIRMINO et al., 2023). In equine pythiosis, in addition to lesions of adjacent structures to the primary lesion, only occasional reports of dissemination to distant organs are available in the literature (REIS JUNIOR et al., 2003; SOUTO et al., 2016). The pulmonary involvement observed in this mare is likely dissemination and not a primary infection, as it is characterized by a focal and well-defined lesion affecting only one pulmonary lobe. Aspiration of detached portions of the nasal lesions may explain the dissemination of the infection to the lung (UBIALI et al., 2013). Although, we could not find evidence of *P. insidiosum* vascular invasion in the present case, hematogenous spread following vascular invasion of hyphae has also been proposed as a route of infection in horses with disseminated pythiosis (REIS JUNIOR et al., 2003).

The clinical manifestation of pythiosis is usually misinterpreted as neoplasia or bacterial infection (SOUTO et al., 2022). The aggressive facial swelling with plenty of mucopurulent discharge in this mare delayed the correct diagnosis and treatment. Indeed, secondary contamination represents an additional obstacle in the diagnosis and treatment of the disease (LEAL et al., 2001). Histomorphology and auxiliary laboratory techniques should be considered to achieve a final diagnosis and exclude other infections frequently associated with rhinitis in horses, such as zygomycosis and phaeoohyphomycosis (MORE et al., 2019). Available laboratory techniques to confirm the diagnoses are microbiological culture, immunohistochemistry, serological assays, and Polymerase Chain Reaction (GAASTRA et al., 2010). Immunohistochemistry has good specificity and sensitivity (MARTINS et al., 2012) and is frequently performed to confirm the diagnosis of *Pythium insidiosum* after histomorphological characterization (MUSTAFA et al., 2015; SOUTO et al., 2016; BIANCHI et al., 2018; SOUTO et al., 2021).

If not treated in the early stages, chronic infections are difficult to resolve and can become life-threatening. Besides the diagnostic obstacles mentioned above, the mare was of low zootechnical value and raised in an extensive system, leading to a

lack of observation of the animal by the owner and, ultimately, causing a delay in seeking veterinarian assistance. Early surgical excision of the lesion is generally associated with good outcomes (SOUTO et al., 2022); however, it would not be a viable option in this case due to the lesion size and risky anatomic region as well as the high cost of the surgery. It is also important to note that this aetiological agent has never been reported in the Department of Tacuarembó and is mostly unknown in that region, which favored the final diagnosis to be accomplished only after post-mortem examination. Triamcinolone acetonide has previously been effectively used to treat cutaneous equine pythiosis in endemic areas of Uruguay (ROMERO et al., 2019).

The disease has been reported mainly in the northwest (SCHANZEMBACH et al., 2019) and eastern (ROMERO et al., 2019) regions of the country in the departments of Treinta y Tres, Rocha, Durazno (ROMERO et al., 2019) and Paysandú (SCHANZEMBACH et al., 2019). The cases occurred in areas of poor or very poor natural drainage (ROMERO et al., 2019), as also observed in our study with adequate characteristics to the proliferation and maintenance of the organism (SCHANZEMBACH et al., 2019). Even though pythiosis is considered an emerging disease in Uruguay (SCHANZEMBACH et al., 2019), studies investigating the spatial epidemiology of the disease revealed optimum environmental characteristics for the occurrence of the disease in the country (MACHADO et al., 2018; ROMERO et al., 2019). The reported cases occurred mainly in areas close to the boundary limits with Brazil; however, ecological niche models have already allowed the identification of larger areas of Uruguay that are suitable for the circulation of *P. insidiosum* beyond the endemic areas even with the risk of transmission and infection in areas with lower temperatures and lower vegetation coverages (MACHADO et al., 2018).

CONCLUSION

Transmission and infection of disseminated equine pythiosis are more broadly distributed throughout Uruguay than previously reported. Our findings suggested that the pulmonary involvement was secondary to the rhinofacial infection rather than a primary site. The lung lesion observed was discrete and restricted to a single pulmonary lobe while the rhinofacial lesions were massive with extensive involvement of adjacent structures and regional lymph nodes, highlighting the chronicity of the clinical condition.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Writing - original draft preparation, CRRQM. Conceptualization, investigation, methodology: CRRQM, MB, CM, YGSS, GJNG, FRC, MM. All authors critically revised the manuscript and approved of the final version.

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