



Case Report

Rhinosporidium seeberi infection in a male mule in the State of Tocantins (Brazil)

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Abstract

Rhinosporidiosis is a chronic disease caused by *Rhinosporidium seeberi*, which can infect several host species, including domestic animals and humans. The taxonomy of *R. seeberi* is still controversial, but scientific evidence supports the classification of this organism as a Mesomycetozoan. Considering the absence of previously reported cases of rhinosporidiosis in the Northern Region of Brazil, the goal of this report was to describe the clinical and pathological findings of a case of *R. seeberi* infection in a male mule in Araguaína (Tocantins State, Brazil). An 8-year-old male mule presented respiratory difficulties due to a polypoid exophytic lesion that partially obstructed the nasal cavity. The lesion was surgically excised and submitted for microscopic analysis. Microscopically, there was multifocal erosion, epithelial hyperplasia, diffuse inflammatory infiltrate, composed of many histiocytes and plasma cells, and fewer neutrophils and lymphocytes, with numerous intralesional organisms with diameters ranging from 10 to 400 µm compatible with sporangia exhibiting numerous nuclei and endoconidia compatible with *R. seeberi*. **Keywords:** rhinosporidiosis, Mesomycetozoan, nasal polyps, rhinitis, granulomatous.

Introduction

Rhinosporidiosis is a chronic disease caused by *Rhinosporidium seeberi*, which may infect various host species, including dogs (12), cats (21), horses (29), mules (6), birds (15), as well as humans (24). Interestingly, the first reported case of animal rhinosporidiosis in Brazil in the 1940s affected a mule (9). *R. seeberi* has virtually a worldwide distribution (10, 16, 29), although there is a comparatively high prevalence in some Asian countries, particularly India and Siri Lanka (8). In Brazil, there are many reported cases of rhinosporidiosis in humans (1, 24). A much smaller number of cases have been reported on

animals in Brazil, including horses (2, 19, 26) and dogs (22), and many of these cases occurred in the State of Maranhão in wet environments (1).

The taxonomy of *R. seeberi* is still controversial, but enough scientific evidence has been accumulating to exclude the hypothesis that this organism is a fungus or a prokaryote; conversely, DNA, phylogenetic, and ultrastructural analyses support the classification of this organism as a Mesomycetozoa. However, it is not currently possible to culture *R. seeberi* so its life cycle is not entirely understood (13, 30). However, it is thought that infection is due to the ability of the organism to invade the skin or mucosa, especially in the nasal cavity (27).



The most common clinical manifestations of *R. seeberi* infection are proliferative polipoid lesions in the nasal cavity that may eventually be associated with epistaxis and occasionally obstruction of the nasal cavity. Less commonly, lesions may be disseminated to the nasopharyngeal mucosa (3, 11). Other mucosae, such as the ocular (23) or preputial (25), may also be rarely affected, as well as subcutaneous lesions in various locations in the body (5).

Considering the absence of previously reported cases of rhinosporidiosis in the North Region of Brazil, the goal of this report is to describe clinical and pathological findings in a case of *R. seeberi* infection of a male mule in Araguaína (State of Tocantins, Brazil).

Case Description

An 8-year-old male mule (a hybrid of male *Equus asinus* and a female *Equus caballus*), which shared the premises with 30 other equids that were all healthy with no clinical changes in a farm located in Araguaína (State of Tocantins, Brazil) developed respiratory distress and intolerance to exercise. Clinical examination evidenced a polypoid exophytic lesion with an irregular surface on the surface of the nasal vestibular mucosa, partially occluding the rostral portion of the left nasal cavity. Surgical therapy was elected, and the nodule was completely removed with some margin of unaffected tissue. The nodular lesion had a soft, clear tan color, measuring 3.1 x 2.0 x 1.7 cm. Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, cut in a microtome (4 µm-thick sections), and stained with hematoxylin and

eosin, Grocott's methenamine silver stain (GMS), or periodic acid–Schiff (PAS).

DNA was extracted from paraffin-embedded tissues using a commercially available kit (Promega DNA Extraction Kit, Promega, USA) according to the manufacturer's instructions. PCR was performed as described (29) using the primers: Rhino 1F (5'-TTTGTGTAGGGGTTCCTCGC-3') and Rhino 1R (5'-GCAAAACCGTTGCTCCAACT-3'). Cycling parameters were 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds.

Microscopically, the polypoid pedunculated tissue had multifocal areas of erosion with a hyperplastic stratified squamous non-keratinized epithelium supported by a proliferating fibrovascular connective tissue (Figure 1), multifocal to coalescing hemorrhage, and a diffuse inflammatory infiltrate composed of many histocytes and plasma cells, and fewer neutrophils and lymphocytes, with numerous large intralesional round organisms with diameter ranging from 10 to 400 µm (Figure 1). There were many juvenile sporangia ranging from 10 to 60 µm with a granular cytoplasm (often not well preserved in the histologic preparation), occasionally seen one or two nuclei, and a thick unilamellar cell wall (Figure 1). There were much less numerous intermediate sporangia with up to 150 µm containing numerous nuclei and a prominent thick bilamellar cell wall (Figure 2). There were also early mature sporangia with more than 150 µm and nuclei that are enclosed in a very thin cell wall; and mature sporangia with numerous endoconidia (Figure 1). Mature sporangia contain numerous fully developed endoconidia containing

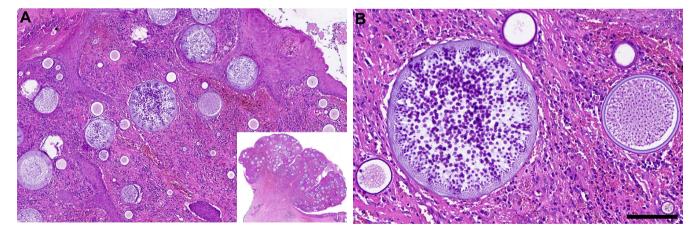


Figure 1. *Rhinosporidium seeberi* infection in a mule. (A) Multifocal areas of erosion and epithelial hyperplasia, diffuse hemorrhage, and inflammatory infiltrate, with numerous large intralesional round organisms with diameters ranging from 10 to 400 μm. Inset: subgross image: pedunculated nodule, supported by fibrovascular connective tissue. Hematoxylin and eosin. (B) Inflammatory infiltrate composed of numerous histiocytes and plasma cells, fewer neutrophils and lymphocytes, and many juvenile sporangia ranging from 10 to 60 μm with a granular cytoplasm and thick unilamellar cell wall, as well as early mature sporangia with more than 150 μm and nuclei that are surrounded by a thin cell wall and mature sporangia with numerous endoconidia. Hematoxylin and eosin. Bar = 500 μm.



Figure 2. *Rhinosporidium seeberi* infection in a mule. Intermediate sporangia, approximately 150 μm in diameter containing numerous nuclei and a prominent thick bilamellar cell wall. Periodic Acid-Schiff (PAS). 40x. Bar = 50 μm.

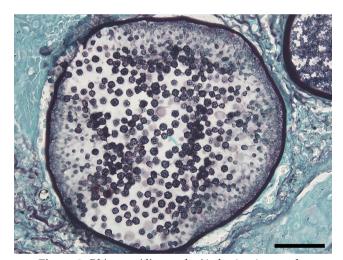


Figure 3. Rhinosporidium seeberi infection in a mule. Mature sporangia of approximately 150 μ m with numerous fully developed endoconidia containing multiple vesicles predominantly in the center and towards the pore and immature sporangia on the margins, with some endoconidia outside the sporangia adjacent to the pore. Grocott's methenamine silver stain (GMS). Bar = 50 μ m.

multiple vesicles predominately at the center and towards the pore and immature sporangia at the margins, with some endoconidia outside the sporangia adjacent to the pore (Figure 3). These morphological features are compatible with *Rhinosporidium seeberi*.

The PCR protocol applied to DNA samples extracted from paraffin-embedded tissues did not yield any amplicon, so DNA sequencing was not achievable in this case.

After the surgery, the horse had an uneventful recovery, with no signs of recurrence up to three years after the surgery.

Discussion

Histologic findings in this case support a conclusive diagnosis of *R. seeberi* infection. Although PCR was attempted but failed to amplify target *R. seeberi* sequences, that does not prevent a conclusive diagnosis since the finding of various stages of typical and very large sporangia is pathognomonic of rhinosporidiosis. Notably, although the first reported case of animal rhinosporidiosis in Brazil was in a mule (9), reports affecting these hybrid animals are quite uncommon. Furthermore, this is the first report of this disease in the North Region of Brazil, which should not be considered unexpected since this case was diagnosed in the North part of the Tocantins State, which is close and somewhat similar to areas of the State of Maranhão where rhinosporidiosis has been previously reported (1).

Considering that culture and isolation of R. seeberi is not doable (4, 20), the diagnosis is based mainly on histopathology since this organism has a distinct microscopic morphology that allows specific identification of the organism. Considering other pathogens that may infect animals and multiply by endosporulation, the closest morphologic differential would be the dimorphic fungi Coccidioides immitis and Coccidioides posadasii whose mature form may reach up to 200 μm, containing many 2-5 μm endospores, and is often associated with a granulomatous inflammatory reaction. However, there are evident morphologic differences between these organisms (including size) and host tissue tropism since disseminated infection often occurs in cases of Coccidioides spp. infection (17). Other endosporulators that infect animals, such as the algae *Prototheca* sp. or *Chlorella* sp., are much smaller, so they cannot be misinterpreted as R. seeberi (28). Ancillary diagnostic methods include cytologic examination (5) and PCR (3, 29); this latter method was unsuccessfully attempted in this case.

In this case, complete exeresis of the lesion was considered curative since there was no recurrence up to three years after surgery, which is still the most effective therapeutic approach in humans (3) and animals (14, 29). However, in some cases, there may be recurrence even many months after surgical removal of the lesion (10). Antimicrobial drugs have been employed for treating rhinosporidiosis, but the efficacy is still limited (7, 18).

In conclusion, we describe here a case of rhinosporidiosis in an adult male mule from the State of Tocantins. To the best of our knowledge, this is the first reported case of this disease in the North Region of Brazil.



Conflict of Interest

The authors declare no competing interests.

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