Braz J Vet Pathol, 2025, vol. 18, e018003
DOI: https://doi.org/10.24070/bjvp.1983-0246.018003

BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Case Report

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

Taismara Simas de Oliveira[1], Silvia de Araújo França Baêta[2], Rodolfo Olinto Rotoli Garcia de Oliveira[3], Antônio Araújo Leite[3], Cristiane América de Carvalho[4], Jefferson Bruno Soares Oliveira[5], Tatiane Alves da Paixão[6], Renato Lima Santos[5]*

[1]Laboratório Hermes Pardini, Belo Horizonte, MG, Brazil
[2]Departamento de Medicina Veterinária, Faculdade de Medicina, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil
[3]Veterinary Practitioners, Araguaína, TO, Brazil
[4]Animale Laboratório Veterinário, Araguaína, TO, Brazil
[5]Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[6]Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

***Corresponding author:** rls@ufmg.br
Submitted: November 13th, 2024. Accepted: February 27th, 2025.

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).

Braz J Vet Pathol, 2025, vol. 18, e018003
DOI: https://doi.org/10.24070/bjvp.1983-0246.018003

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 histiocytes 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

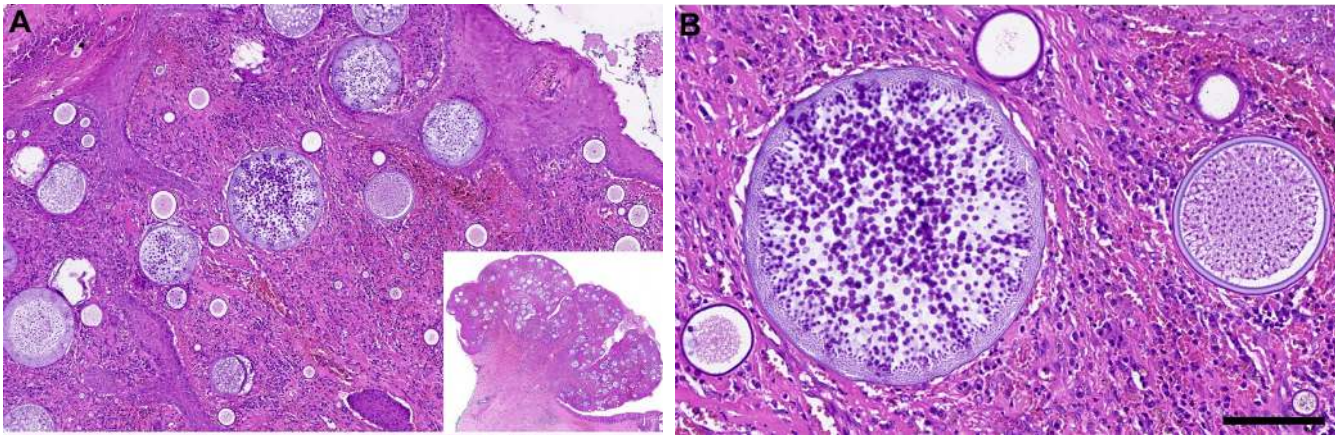

Figure 1. *Rhinosporidium seeberi* infection in a mule. (A) Multifocal areas of erosion and epithelial hyperplasia, diffuse hemorrhage, and inflammatory infiltrate, with numerous large intralesional round organisms with diameters ranging from 10 to 400 µm. Inset: subgross image: pedunculated nodule, 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.

Braz J Vet Pathol, 2025, vol. 18, e018003
DOI: https://doi.org/10.24070/bjvp.1983-0246.018003

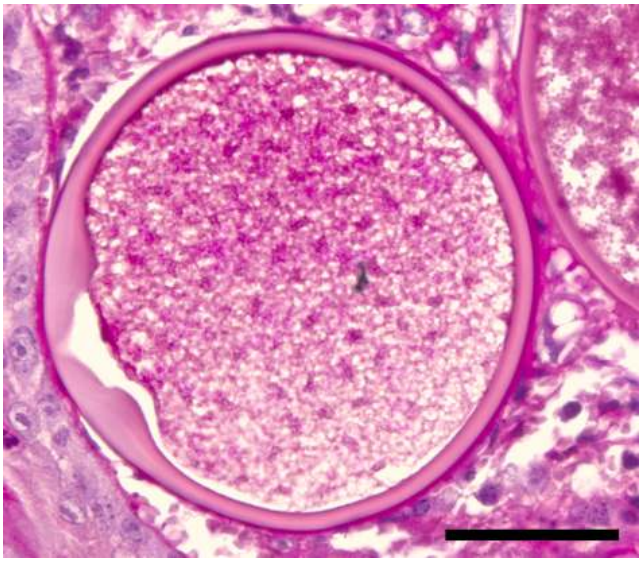

Figure 2. *Rhinosporidium seeberi* infection in a mule. Intermediate sporangia, approximately 150 µm in diameter containing numerous nuclei and a prominent thick bilamellar cell wall. Periodic Acid-Schiff (PAS). 40x. Bar = 50 µm.

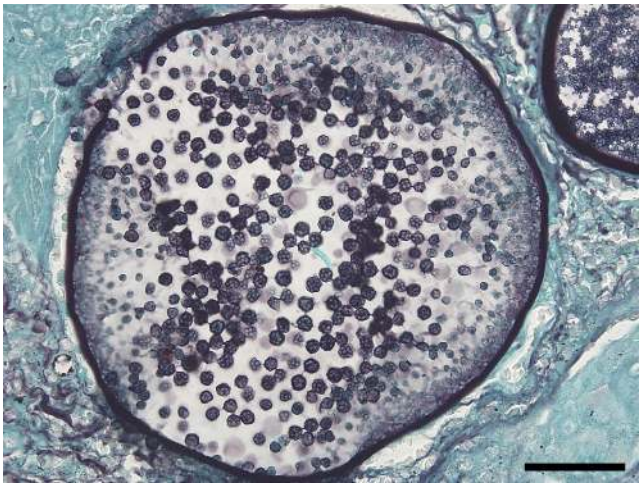

Figure 3. *Rhinosporidium seeberi* infection in a mule. Mature sporangia of approximately 150 µm with numerous fully developed endoconidia containing multiple vesicles predominantly in the center and towards the pore and immature sporangia on the margins, with some endoconidia outside the sporangia adjacent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

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.

Braz J Vet Pathol, 2025, vol. 18, e018003
DOI: https://doi.org/10.24070/bjvp.1983-0246.018003

Conflict of Interest

The authors declare no competing interests.

Acknowledgments

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil), FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Brazil), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil).

References

1. Almeida FA, Feitoza LM, Pinho JD, Mello, GCF, Lages JS, Silva FF, Silva RR, Silva GEB. Rhinosporidiosis: the largest case series in Brazil. Rev Soc Bras Med Trop. 2016; 49(4):473-76. doi:10.1590/0037-8682-0193-201.
2. Argenta FF, Mello LS, Vielmo A, Pavarini SP, Driemeier D, Sonne L. Rhinosporidiosis in horses. Pesq Vet Bras. 2018;38(12):2213-16. Doi: 10.1590/1678-5150-PVB-5982.
3. Arias AF, Romero SD, Garcés CG. Case Report: Rhinosporidiosis Literature Review. Am J Trop Med Hyg. 2021;104(2):708-11. doi: 10.4269/ajtmh.20-0291.
4. Arseculeratne SN. Rhinosporidiosis: what is the cause? Curr Opin Infect Dis. 2005; 18(2):113-8. doi: 10.1097/01.qco.0000160898.82115.e8.
5. Balasubramaniam VV, Ramamoorthi S, Nadeem N, Sekar MD, Gochhait D, Siddaraju N. Disseminated rhinosporidiosis masquerading as soft tissue round cell tumour diagnosed by fine needle aspiration cytology. Cytopathology. 2023;34(1):77-81. doi: 10.1111/cyt.13183.
6. Berrocal A, López A. Nasal rhinosporidiosis in a mule. Can Vet J. 2007;48:305-06.
7. Bhat V. Comments on novel multidrug therapy for disseminated rhinosporidiosis, refractory to dapsone - case report. Trop Doct. 2014;44(1):59-60. doi: 10.1177/0049475513512635.
8. Branscomb R. Rhinosporidiosis Update. Lab Med. 2002;33(8):631–33, https://doi.org/10.1309/4Q3P-X7QX-VVX9-RKHK.
9. Bueno P, Faria, PN. Rinosporidiose em muar. Arq Inst Biol. 1941;12 (20):297–302.
10. Burgess HJ, Lockerbie BP, Czerwinski S, Scott M. Equine laryngeal rhinosporidiosis in western Canada. J Vet Diagn Invest. 2012;24(4):777-80. doi: 10.1177/1040638712445773.
11. Chandran A, Sakthivel P, Kumar P, Rajeshwari M. Pharyngeal rhinosporidiosis. BMJ Case Rep. 2020;13(6): e236404. doi: 10.1136/bcr-2020-236404.
12. Cridge H, Mamaliger N, Baughman B, Mackin AJ. Nasal Rhinosporidiosis: Clinical Presentation, Clinical Findings, and Outcome in Dogs. J Am Anim Hosp Assoc. 2021;57(3):114-120. doi: 10.5326/JAAHA-MS-7121.
13. Herr RA, Ajello L, Taylor JW, Arseculeratne SN, Mendoza L. Phylogenetic analysis of *Rhinosporidium seeberi's* 18S small-subunit ribosomal DNA groups this pathogen among members of the protoctistan Mesomycetozoa clade. J Clin Microbiol. 1999;37(9):2750-4. doi: 10.1128/JCM.37.9.2750-2754.1999.
14. Hill AS, Sharkey LC, Hardy RM, Wilke VL, Smith MA, Anderson GM. Nasal rhinosporidiosis in two dogs native to the upper Mississippi river valley region. J Am Anim Hosp Assoc. 2010;46(2):127-31. doi: 10.5326/0460127.
15. Kennedy FA, Buggage RR, Ajello L. Rhinosporidiosis: a description of an unprecedented outbreak in captive swans (*Cygnus* spp.) and a proposal for revision of the ontogenic nomenclature of *Rhinosporidium seeberi*. J Med Vet Mycol. 1995;33(3):157-65. doi: 10.1080/02681219580000341.
16. Leeming G, Smith KC, Bestbier ME, Barrelet A, Kipar A. Equine rhinosporidiosis in United Kingdom. Emerg Infect Dis. 2007;13(9):1377-9. doi: 10.3201/eid1309.070532.
17. Macías-Rioseco M, Sheley M, Ochoa J, Carvallo-Chaigneau FR, Uzal FA. Coccidioidomycosis in 26 horses in California, USA: case series and review of the literature. J Vet Diagn Invest. 2022;34(6):995-999. doi: 10.1177/10406387221114622.
18. Madke B, Mahajan S, Kharkar V, Chikhalkar S, Khopkar U. Disseminated cutaneous with nasopharyngeal rhinosporidiosis: Light microscopy changes following dapsone therapy. Australas J Dermatol. 2011;52(2):e4-6. doi: 10.1111/j.1440-0960.2010.00633.
19. Matias IC, Soares LA, Gurgel AC, Cordeiro AE, Silva CG, Oliveira Filho HS, Bezerra HMFF, Sucupira Júnior G, Galiza GJN, Maia LA, Dantas AFM. Epidemiological, clinical and anatomopathological characterization of nasal rhinosporidiosis in a horse. Res Soc Dev. 2022;11(12): e99111234075. doi: http://dx.doi.org/10.33448/rsd-v11i12.34075.
20. Mendoza L, Taylor JW, Ajello L. The class mesomycetozoea: a heterogeneous group of microorganisms at the animal-fungal boundary. Annu Rev Microbiol. 2002;56:315-44. doi: 10.1146/annurev.micro.56.012302.160950.
21. Moisan PG, Baker SV. Rhinosporidiosis in a cat. J Vet Diagn Invest. 2001;13(4):352-4. doi: 10.1177/104063870101300414.
22. Neves CD, Dutra NR, Berbari Neto F, Silveira LS. Canine nasal rhinosporidiosis: case report in Brazil. R bras Ci Vet. 2014;21(4):223-5. doi: http://dx.doi.org/10.4322/rbcv.2015.299.
23. Parida PK, Thangavel S, Raja K, Saxena SK. Lacrimal sac rhinosporidiosis. BMJ Case Rep. 2021;14(6):e243926. doi: 10.1136/bcr-2021-243926.

24. Penagos S, Zapata N, Castro JJ, Hidron A, Agudelo CA. Rhinosporidiosis in the Americas: A Systematic Review of Native Cases. Am J Trop Med Hyg. 2021;105(1):171-175. doi: 10.4269/ajtmh.20-1411.
25. Saiduzzaman BM, Chakravarty S, Rahman MDS, Haider M, Islam MDM, Mahmud MDS. Urethral rhinosporidiosis: Prolapsing mass from urethra in a Bangladeshi farmer. IDCases. 2023;33:e01825. doi: 10.1016/j.idcr.2023.e01825.
26. Santos AC, Nogueira CEW, Vieira PS, Araujo LO, Amaral LA, Pazinato FM, Santos FCC, Curcio BR. Rinosporidiose nasal e laringeana em equino. Acta Sci Vet. 2014;42(62):1-5.
27. Sinha A, Phukan JP, Bandyopadhyay G, Sengupta S, Bose K, Mondal RK, Choudhuri MK. Clinicopathological study of rhinosporidiosis with special reference to cytodiagnosis. J Cytol. 2012;29(4):246-9. doi: 10.4103/0970-9371.103943.
28. Souza LN, Estrela-Lima A, Moreira ELT, Ribeiro LGR, Xavier MN, Silva TMA, Costa EA, Santos RL. Systemic canine protothecosis. Braz J Vet Pathol. 2009;2: 102-6.
29. Tizzano MA, Della Vedova R, Lopez RA, Amor V, Zubía C, Córdoba SB, Reynaldi FJ. Equine rhinosporidiosis in Buenos Aires, Argentina. Rev Argent Microbiol. 2022;54(1):22-4. doi: 10.1016/j.ram.2020.12.007.
30. Vilela R, Mendoza L. The taxonomy and phylogenetics of the human and animal pathogen *Rhinosporidium seeberi*: a critical review. Rev Iberoam Micol. 2012;29(4):185-99. http://dx.doi.org/10.1016/j.riam.2012.03.012.