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PATHOLOGY

Granulomatous rhinitis by *Neoconidiobolus lamprauges* in a mule

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ABSTRACT: *Conidiobolomycosis has a wide distribution, predominantly in humid tropical regions, affecting several species with significant mortality rates. The genus Conidiobolus is now divided into four genera: Capillidium, Conidiobolus, Microconidiobolus, and Neoconidiobolus. There are no confirmed reports of infection by these fungi in Equidae in Brazil. We present a rhinofacial rhinitis caused by Neoconidiobolus lamprauges in a mule from Rio de Janeiro, Brazil. The mule presented bilateral semi-occlusion of the nostrils, difficulty breathing, and weight loss. The histological examination of the nostril biopsied mass revealed multifocal necrotizing areas with nonstained images of fungal hyphae in the Splendore-Hoeppli reaction and surrounded by macrophages, eosinophils, neutrophils, and multinucleated giant cells. The Grocott methenamine silver staining revealed thin-walled, rarely septated, irregular branching hyphae, with a varying diameter of 12 µm (± 3.63 µm), and terminal ballooning dilations. The determining etiology of this rhinitis was based on the hyphae staining by immunohistochemistry and by amplifying the DNA fragment of N. lamprauges by polymerase chain reaction. Conidiobolomycosis should be included in the differential diagnosis of the causes of rhinitis in equids, mainly in tropical regions.*
Key words: *equids, Zygomycetes, fungal infection, granulomatous rhinitis, conidiobolomycosis, immunohistochemistry, molecular analysis.*

Rinite granulomatosa por *Neoconidiobolus lamprauges* em uma mula

RESUMO: *A conidiobolomicose apresenta ampla distribuição geográfica, com predominância em regiões tropicais úmidas, afeta várias espécies e apresenta taxa de mortalidade significativa. O gênero Conidiobolus está agora dividido em quatro gêneros: Capillidium, Conidiobolus, Microconidiobolus e Neoconidiobolus. Não há relatos confirmados de infecção por esses fungos em equídeos no Brasil. Relata-se o caso de uma mula com rinite rinofacial causada por Neoconidiobolus lamprauges no Rio de Janeiro, Brasil. A mula apresentava uma massa que semiocluía as narinas bilateralmente, dificuldade respiratória e emagrecimento. O exame histológico de biópsia da massa da narina revelou áreas de necrose multifocais com imagens negativas de hifas fúngicas em meio à reação de Splendore-Hoeppli, circundadas por macrófagos, eosinófilos, neutrófilos e células gigantes multinucleadas. O exame histoquímico metenamina prata de Grocott revelou hifas de parede fina, raramente septadas, com ramificação irregular, grau variável de paralelismo, e diâmetro médio de 12 µm (± 3,63) e dilatações balonosas terminais. O diagnóstico etiológico foi realizado pela associação da imuno-histoquímica e da amplificação do fragmento de DNA de N. lamprauges pela reação em cadeia da polimerase. A conidiobolomicose deve ser incluída no diagnóstico diferencial das causas de rinite em equídeos, principalmente em regiões tropicais.*
Palavras-chave: *equídeos, Zygomycota, infecção fúngica, rinite granulomatosa, conidiobolomicose, imuno-histoquímica, análise molecular.*

Conidiobolomycosis is a disease caused by the former genus *Conidiobolus*, saprobic fungi of the class Zygomycetes (VILELA & MEDONZA, 2018). Three pathogenic species were described, *C. lamprauges*, *C. coronatus,* and *C. incongruus* (VILELA et al., 2010). The genus *Conidiobolus* was divided by morphological and molecular methods into four genera: *Capillidium*, *Conidiobolus*, *Microconidiobolus*, and *Neoconidiobolus* (NIE et al., 2021). These fungi mainly affect the nasal mucosa, subcutaneous tissue or the skin (VILELA & MEDONZA, 2018). Infection occurs by inhaling

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spores or microlesions by insects or pointed plants that allow direct inoculation of spores (KETTERER et al., 1992; SILVA et al., 2007; NIE et al., 2021).

The disease has a wide distribution and occurs predominantly in subtropical and tropical regions with a humid climate. Both naturally occurring (MORE et al., 2019; CARMO et al., 2021) and experimental infection showed high mortality rates (GODOY et al., 2017). Mammal species, including humans (PESTANA et al., 2019), sheep (CARMO et al., 2021), goats (MACÊDO et al., 2021), canines (JAFFEY et al., 2021), llamas (FRENCH & ASHWORTH, 1994), deer (STEPHENS & GIBSON, 1997), and horses (MORE et al., 2019) may be affected by different species of *Conidiobolus*. In sheep, conidiobolomycosis has been described in Brazil (BOABAID et al., 2008; RIET-CORREA et al., 2008; FURLAN et al., 2010) in multiple geographic regions, considered endemic with morbidity of up to 6% and lethality of 100% (CARMO et al., 2021).

Clinical signs of conidiobolomycosis in equines are restricted to the respiratory system. The main clinical signs are unilateral (TAINTOR et al., 2004) or bilateral epistaxis (STEIGER & WILLIAMS, 2000), unilateral mucopurulent (KORENEK et al., 1994), or mucopurulent and hemorrhagic exudate (MORE et al., 2019). Respiratory sounds and coughing occur due to the presence of inflammatory masses in the nasal septum (KORENEK et al., 1994; HUMBER et al., 1989), nasopharynx (TAINTOR et al., 2004; ZAMOS et al., 1996), larynx (MILLER & CAMPBELL, 1984) and trachea (STEIGER & WILLIAMS, 2000). The diagnosis of conidiobolomycosis can be confirmed by isolating the agent in culture, immunohistochemical detection (UBIALI et al., 2013) or molecular techniques such as polymerase chain reaction (PCR) (VILELA et al., 2010; SILVEIRA et al., 2013). Surgery or administration of antifungal drugs are treatments related to variable success for treating conidiobolomycosis in horses (ZETTERSTROM et al., 2020).

We described the clinic-pathological findings of a confirmed case of nasal conidiobolomycosis in a mule in Brazil, intending to alert veterinarians about this respiratory disease.

In October 2018, a nodule fragment from an incisional biopsy of a bilateral intranasal lesion of a 13-year-old mule from the municipality of Mangaratiba, coastal region of Rio de Janeiro, Brazil (S 22 57 35, W 44 2 28), was sent to the *Setor de Anatomia Patológica* (SAP) of the Federal Rural University of Rio de Janeiro (UFRuralRJ) for diagnosis.

Clinical and epidemiological data were obtained from the veterinarian who sent the lesion biopsied for diagnosis. The sedation protocol was administered by application of xylazine. After the mule reached the desired sedation plan, an incision on the lesion was done, and the obtained tissue was fixed in 10% buffered formalin. Finally, silver sulfadiazine and cypermethrin repellent were applied to the lesion site. Nasal tissues were routinely processed for histology and stained with hematoxylin and eosin and Grocott methenamine silver (GMS) and evaluated microscopically. The hyphae's diameter was determined from the GMS stained sections (MILLER & CAMPBELL, 1984).

The 3µm sections of the nodule were subjected to immunohistochemistry (IHC) using polyclonal antibodies anti-*C. lamprauges* and anti-*Pythium insidiosum*. The antibodies were raised by immunizing rabbits with *C. lamprauges* exoantigens from purified cultures; or *P. insidiosum* after subcutaneous inoculation of zoospores (UBIALI et al., 2013). The isolates used to develop the antigens for the production of the anti-*C. lamprauges* antibodies were recorded by SILVEIRA et al. (2013) (GenBank GQ478281.1). Antigen retrieval was performed with citrate buffer (10 mM, pH 6.0) at 96 ºC for 20 min. Both primary antibodies were used at a concentration of 1:1000, incubated at 38 ºC for two hours. The secondary antibody used was streptavidin-biotin-peroxidase (LSAB + System HRP, Agilent Technologies, Santa Clara, CA, USA). Substrate development occurred due to the addition of 3,3-diaminobenzidine chromogen (DAB + Substrate Chromogen System, DakoCytomation, Carpinteria, California). The sections were contrasted with Mayer's hematoxylin. During the test, tissues previously cultured and PCR positive (conidiobolomycosis from sheep) and negative controls (replacement of primary antibody by phosphate buffer saline and normal nasal tissue) were used simultaneously.

Nasal lesion tissue, formalin-fixed paraffin-embedded, was sent to the Molecular Biology Laboratory. The DNA was extracted according to SHI et al. (2004), and it was subjected to the PCR technique with oligonucleotides based on the 18S ribosomal gene, which amplified 222 bp, specific for the identification of *C. lamprauges* (SILVEIRA et al., 2013). Subsequently, the DNA was subjected to the PCR technique with the oligonucleotide pair encoding the sequence of the ITS1 rDNA gene of *P. insidiosum*, which amplifies 105 bp, specific for the identification of *P. insidiosum* (AZEVEDO et al., 2012). Positive controls for conidiobolomycosis

and pythiosis were nasal and cutaneous lesions from sheep and horse, respectively, previously diagnosed by culture and PCR.

The mule presented bilateral semi-occlusion of the nostrils, difficulty breathing, and weight loss. These clinical signs were due to inflammatory masses that invaded the alar fold and dorsal meatus and partially occluded both nostrils. The mass was firm and measured approximately 5.3 x 4.2 x 3.0 cm, with an irregular surface, with multifocal brown and yellow areas. A moderate bilateral increase of submandibular lymph nodes was also observed.

The biopsied nostril mass was 2.5 x 1.2 x 1.2 cm, in its largest axes, with an irregular surface, and the cut surface was firm, compact with multifocal necrotic brown-yellow areas (Figure 1A). The histological evaluation demonstrated the mucocutaneous nasal mass with ulceration, multifocal areas of necrosis containing eosinophilic clots (Splendore-Hoeppli reaction) and non-stained images of fungal hyphae, a significant amount of macrophages, a moderate number of multiple giant cells (Figure 1B), eosinophils, and neutrophils, fibrosis, and neovascularization. The histochemistry with GMS showed round tubuliform hyphae, with a varying degree of parallelism and diameter average of 12 µm (± 3.63 µm), with thin walls and terminal balloon dilations (Figure 1C).

The IHC and the PCR for *C. lamprauges* revealed intense immunolabelling of intralesional hyphae (Figure 1D) and the amplicon of 222 bp, respectively. The primers are putatively specific for *N. lamprauges*. After a clinical evolution of five months in the field, the mule died, and the necropsy was not undergone.

The diagnosis of conidiobolomycosis was based on clinical respiratory signs due to bilateral

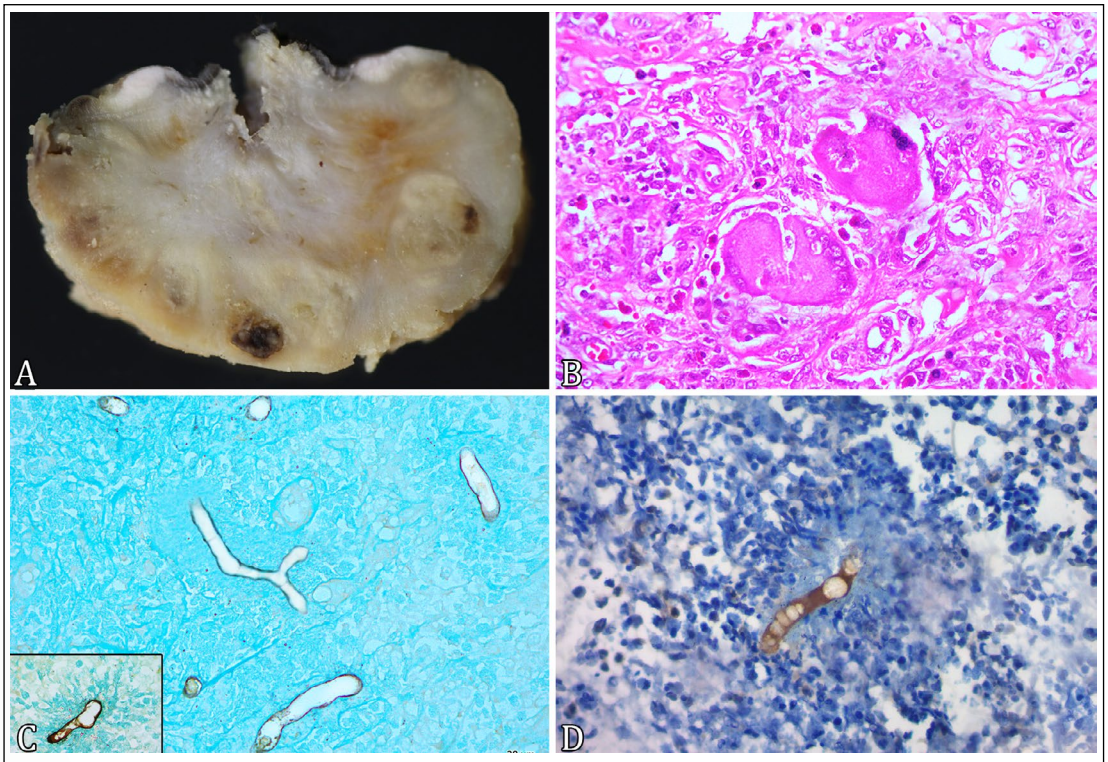

Figure 1 - Mule, nasal conidiobolomycosis. (A) Mucocutaneous intranasal mass section with multifocal yellow-brown necrotic areas at the cut surface. (B) Nasal tissue with macrophages and non-stained images of fungal hyphae within multinucleated giant cells (HE, 63X). (C) Tubuliform thin-walled, rarely septated, irregular branching hyphae (GMS, 63X). The inset demonstrates hyphae terminal dilations (D). Intralesional hyphae revealed intense immunolabelling for antibodies against *Conidiobolus lamprauges* (IHC Hematoxylin counterstain, 63X).

nostrils semi-occlusion by an inflammatory mass, histological pattern, and confirmed by IHC and PCR. The testing of *P. insidiosum* by IHC and PCR was negative. We emphasized the importance of auxiliary methods such as IHC (UBIALI et al., 2013) and PCR (VILELA et al., 2010) as valuable techniques in providing a quick and accurate diagnosis compared to mycological cultures, which generally require more time and experience from the mycologist. The genus *Conidiobolus* was reclassified into four genera (*Capillidium, Conidiobolus, Microconidiobolus,* and *Neoconidiobolus*) (NIE et al., 2021). Based on the author's experience, it is complicated to distinguish among them without molecular testing.

The lesion of the present report was rhinofacial bilateral, affecting the rostral nasal cavity. In horses, the injury caused by the former *Conidiobolus* spp. is predominantly located in the nasal cavity. Lesions can be rhinofacial, restricted to the nasal cavity (MORE et al., 2019; MILLER & CAMPBELL, 1984) or anatomic located in the guttural sac, nasal septum (TAINTOR et al., 2004), nasal concha (KORENEK et al., 1994), nasopharynx (HUMBER et al., 1989; ZAMOS et al., 1996; MILLER & CAMPBELL, 1984) or trachea (STEIGER & WILLIAMS, 2000).

Only one case of conidiobolomycosis in a mule was reported in the literature (CARVALHO et al., 1976). However, those authors based only on histomorphological patterns and did not present diagnostic confirmation through etiological exams. The current case confirmed the susceptibility of mules to infection by *Neoconidiobolus lamprauges* and expands the range of host species known for conidiobolomycosis. There are no confirmed reports of *Conidiobolus* spp. infection in Equidae in Brazil.

An important differential diagnosis of nasal conidiobolomycosis in horses is nasal pythiosis (SOUTO et al., 2016). When comparing both diseases, in a 35-year-study, the author's findings revealed that 195 horses, six mules, and one donkey were diagnosed with pythiosis (SOUTO et al., 2021). A research in the Brazilian Pantanal showed that horses were more affected (97.4%) by pythiosis than domestic equine hybrids (2.6%) (SANTOS et al., 2014). A resistance of equid hybrids by oomycete or fungi infection compared to equine is a hypothesis. Conversely, Brazil has different regions with heterogeneous populations of horses, mules and donkeys. Therefore, we encouraged other groups to compare host-pathogen interaction in horses and their hybrids.

The diseases of the nasal cavity of horses present similar clinical signs, characterized mainly by nasal discharge, epistaxis and breathing difficulties (NICKELS, 1993). As a result, the differential diagnoses of rhinitis causes in horses must be differentiated of *Basidiobolus ranarum* (JOHNSON et al., 2021), *Rhinosporidium seeberi* (ARGENTA et al., 2018), *P. insidiosum* (SOUTO et al., 2016; TONPITAK et al., 2018), *Alternaria* spp., *Pseudallescheria boydii* (MORE et al., 2019) and *Cryptococcus* spp. (CRUZ et al., 2017). Other conditions that affect the nasal cavity of horses include congenital nasal cysts, inflammatory polyps, nasal amyloidosis, neoplasms, nasal granulomas caused by hypersensitivity and progressive ethmoidal hematoma (MORE et al., 2019; NICKELS, 1993).

In a survey conducted in Florida, in the United States, with horses that presented mycotic rhinitis and sinusitis, the diagnostic rate for conidiobolomycosis was 62% (32/51), which corresponds to the largest group of lesions (MORE et al., 2019). This high frequency represents the involvement of the nasal mucosa by saprophytic fungal spores (MANNING et al., 2007). The hypotheses on animal and human contamination are dust inhalation, nasal mucosa contact with contaminated water, traumatic injuries by plants with thorns and exposure to insects that transport different conidia species (KETTERER et al., 1992; VILELA et al., 2010, VILELA & MENDONZA, 2018, CARMO et al., 2021). It was not possible to determine the field infection source in this mule with conidiobolomycosis.

Conidiobolomycosis should be included in the differential diagnosis of the causes of rhinitis in equids, mainly in tropical regions. We emphasized the diagnostic importance through histology, immunohistochemistry, and molecular identification.

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DECLARATION OF CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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