



INFECTIOUS DISEASE

Pythiosis in the Nasal Cavity of Horses

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Summary

Two cases of nasal pythiosis are reported in horses from the semi-arid region of northeastern Brazil. From January 1986 to December 2015, the Laboratory of Animal Pathology, Federal University of Campina Grande received 830 equine samples, 156 (18.79%) of which were diagnosed with pythiosis. Of these, two horses (1.28%), a male and a female adult cross-breed, had lesions in the nasal cavity. Both horses had access to water reservoirs. Clinically, they had swelling in the rhinofacial region and a serosanguineous nasal discharge. Macroscopically, in case 1, the lesion affected the nasal vestibule, extending to the alar cartilage and nasal septum. In case 2, the lesion extended through the turbinates and the meatuses of the nasal cavity, as well as the ethmoid region. In both cases, the lesions were characterized by having a yellow–grey granular surface with cavitations of different sizes containing coral-like masses of necrotic tissue (kunkers). Histologically, multifocal necrotizing eosinophilic rhinitis associated with hyphae (2–8 μm) similar to *Pythium insidiosum* were observed. In case 2, the lesions extended to the muscle, cartilage and bone adjacent to the nasal cavity and lungs. The diagnosis was confirmed by immunohistochemistry. It is concluded that nasal pythiosis occurs sporadically in horses in the semi-arid region of northeastern Brazil where cutaneous pythiosis is prevalent.

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Pythiosis is a chronic inflammatory disease caused by the oomycete *Pythium insidiosum* (kingdom Stramenopila, family Pythiaceae) that affects man and animals (Chaffin *et al.*, 1995). The clinical form of the disease can vary accordingly to the entry site of the agent and the affected animal species (Gaastra *et al.*, 2010).

In horses, pythiosis has been observed most frequently in the skin (Chaffin *et al.*, 1995; Tabosa *et al.*, 1999; Leal *et al.*, 2001; Sallis *et al.*, 2003; Martins *et al.*, 2012; Pessoa *et al.*, 2014); however, intestinal (Brown and Roberts, 1988; Allison and Gillis, 1990; Morton *et al.*, 1991; Purcell *et al.*, 1994) and pulmonary forms (Goad, 1984) are also recorded. The cutaneous form may involve the lymph nodes

(Chaffin *et al.*, 1995; Martins *et al.*, 2012) and bones (Mendoza *et al.*, 1988; Alfaro and Mendoza, 1990; Eaton, 1993).

Nasal pythiosis has been described in sheep (Riet-Correa *et al.*, 2008; Santurio *et al.*, 2008; Portela *et al.*, 2010) and cats (Bissonnette *et al.*, 1991) and although nasal lesions in a horse were mentioned in a retrospective study in Rio Grande do Sul, Brazil (Galiza *et al.*, 2014), the clinical and pathological features of this clinical form have not been reported previously. The purpose of this study is to report two cases of nasal pythiosis in horses in Brazil.

A retrospective study of all biopsy samples and necropsy examinations of horses, from January 1986 to December 2015, at the Animal Pathology Laboratory of the Federal University of Campina Grande, Patos,

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Brazil, was carried out. Among the cases of pythiosis, only those that involved the nasal cavity were selected. Epidemiological data, clinical signs and gross lesions were reviewed from the reports. Samples of the nasal lesions, lymph nodes, central nervous system and organs of the thoracic and abdominal cavities were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE) and selected sections were also stained with Grocott's methenamine silver stain (GMS).

Immunohistochemistry (IHC) was performed for identification of the agent in samples of the nasal cavity lesions in both cases. The IHC protocol was performed according to Martins *et al.* (2012) using a polyclonal antibody (anti-*P. insidiosum*) produced in rabbits (Gabriel *et al.*, 2008). Briefly, the primary antibody at a dilution of 1 in 1,000 was detected by use of streptavidin–biotin–alkaline phosphatase complex and labelling was ‘visualized’ with Permanent Red (Dako, Glostrup, Denmark) as a substrate–chromogen. We analyzed a previously confirmed positive control (equine cutaneous pythiosis) simultaneously with the tested samples. The negative control included tissue samples incubated with phosphate buffered saline instead of primary antibody.

During the study period, we received 830 tissue samples from necropsy examinations and biopsy sampling in horses. Among these samples, 156 (18.79%) had lesions of pythiosis. Of these, 154 cases (98.72%) corresponded to the cutaneous/subcutaneous form of the disease and in two cases (1.28%), the lesions were located in the nasal cavity. Case 1 was a 25-year-old mixed breed horse from the city of Santa Terezinha, Paraíba. This animal was taken to the Veterinary Hospital in June 2010 with severe swelling of the nostrils with an ulcerated central area and a serosanguineous discharge. Because of the severe clinical condition and poor prognosis, the animal was humanely destroyed. Case 2 was a 14-year-old mixed-breed mare from the city of Patos, Paraíba. This horse was taken to the Veterinary Hospital in August 2011 with progressive weight loss, dehydration, dyspnoea, facial swelling and a fetid nasal wound with a serosanguineous discharge, conditions that led to humane destruction of the horse.

The heads from the two cases were sectioned longitudinally along the midline. In case 1, the lesion (approximately 10 cm diameter) affected the entire nasal vestibule and extended to the alar cartilage and the nasal septum. It had a yellow–grey, dark, irregular cut surface with cavities of different sizes, containing coral-like necrotic tissue (kunkers) (Fig. 1). Case 2 involved bilateral compression and replacement of the turbinate bones and meatuses of



Fig. 1. Case 1. Areas of necrosis and cavitation containing kunkers in the nasal vestibule.

the nasal cavity by a multifocal to coalescing yellow–grey, irregular and firm mass approximately 18 cm in diameter (Fig. 2), with cavities containing coral-like necrotic tissue. Another similar lesion measuring approximately 6 cm in diameter was observed in the ethmoid region (Fig. 2).

Histological lesions in both horses were multifocal to coalescing areas of eosinophilic necrosis surrounded predominantly by eosinophils (horse 1) or neutrophils (horse 2), with epithelioid cells, multinucleated giant cells, lymphocytes and plasma cells (Fig. 3). Adjacent to these areas there was extensive fibroplasia with neovascularization, infiltrated by eosinophils and sometimes associated with haemorrhage. Within the necrotic foci, negatively stained hyphae were observed in HE-stained sections. The hyphae, morphologically similar to those of oomycetes,

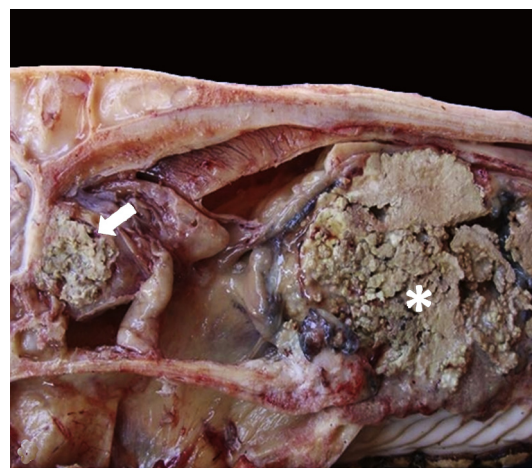


Fig. 2. Case 2. Necrotic areas with high concentrations of kunkers in the nasal turbinates, meatuses (asterisk) and ethmoid region (arrow).

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