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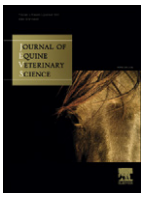

Case Report

Cryptococcal Pneumonia and Meningitis in a Horse

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ABSTRACT

Gross and microscopic evidence of *Cryptococcus neoformans* in the lungs and central nervous system of a mature Thoroughbred horse presenting with granulomatous pneumonia and meningitis has been described in this article.

1. Introduction

Cryptococcus neoformans is a type of saprophytic yeast that sporadically causes disease. It is found chiefly in pigeon (*Columba livia*) feces, and is most frequently acquired through airborne transmission by inhalation, which may lead to opportunistic infection in immunocompromised animals and people [1,2]. In human cases, the majority are meningitis preceded by asymptomatic or symptomatic pulmonary infection [2].

C neoformans causes a systemic fungal disease affecting various organs, mainly those of the respiratory system (nasal cavity and lung) and the central nervous system (CNS) [1], with particular affinity for the latter. It reaches the CNS by leukocytic trafficking and hematogenous spread from primary sites of infection in other areas (lung) of the body [3]. In horses, the infection is uncommon but sporadic cases have been associated with granulomatous pneumonia [4-8], nasal granuloma [9-11], endometritis and placentitis with neonatal cryptococcal pneumonia [12], abortion [13,14], mesenteric lymph node abscess [6], intestinal polypoid granulomas [15], and osteomyelitis [16]. *Cryptococcus* has been described as a cause of fatal meningitis in horses [17-19]. Cho et al. [20] described extensive gross and microscopic cavitary lesions of cerebral cryptococcosis in a mare with encephalitis that presented with marked depression and ataxia.

C neoformans is the most common cause of granulomatous pneumonia in animals. Cryptococcosis causes a systemic fungal disease, and the portal of entry is aerogenous, and from the lungs the fungi disseminate to other organs. The granulomatous pneumonia is characterized by the presence of variable numbers of caseous or noncaseous granulomas randomly distributed in the lungs. On palpation, lungs have a typical nodular character given by well-circumscribed, variably sized nodules that, generally, have a firm texture, especially if calcification has occurred. Microscopically, pulmonary granulomas are composed of a center of necrotic tissue, surrounded by macrophages (epithelioid cells) and giant cells, and an outer delineated layer of connective tissue commonly infiltrated by

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lymphocytes and plasma cells. The causative agent may be identified microscopically in sections tissues [21].

The pathogen can reach the CNS most often from a pulmonary infection site, or by entering the leptomeninges and subarachnoid space by direct extension through the cribriform plate following a nasal or sinus infection, or by leukocytic trafficking and hematogenous spread. It secretes a thick protective mucopolysaccharide capsule, the accumulation of which in the leptomeninges leads to a cloudy to viscous appearance. Microscopically, leptomeningeal lesions have a loosely organized, lacy appearance within which cryptococcal organisms are visible. The response can vary from sparse to granulomatous inflammation, and the leukocytic response consists of proliferation of neutrophils, eosinophils, macrophages, giant cells, and small mononuclear cells, depending on the immune status of the host [3].

The yeast is spherical (2 to 10 μm in diameter), usually surrounded by a thick mucoid capsule (1 to 30 μm diameter) that does not stain with hematoxylin and eosin (H & E). It reproduces by narrow-based buds. Special stains such as periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS) readily identify the yeast, and the capsule can be stained with mucicarmine and alcian blue [3].

Clinically, cryptococcosis with CNS involvement occurs in cats, dogs, horses, and cattle. The neurologic signs vary with the location of the lesions, but can include depression, ataxia, seizures, paresis, and blindness [3].

There are no specific measures for preventing the disease. It is important to avoid long-term corticosteroid treatment against subjacent pathologies because cryptococcosis is recognized as an opportunistic infection in immunocompromised animals [1,2]. Pigeon population control may reduce transmission of the pathogen. Animal facilities must be kept clear of nests and feces [2].

2. Case Report

A case of cryptococcal granulomatous pneumonia and meningitis has been described in a mature Thoroughbred horse stabled at an equine racing center located in an urban area of Brazil. Pigeon nests and feces were observed in the ledges and in the stable roofs. The pigeons usually ate the remains of equine food (seeds, grains) from the feed troughs, and defecated in the feed and water trough.

The horse initially presented with respiratory symptoms of approximately 1 month of duration, including high respiratory rate, abdominal breathing, sporadic cough and fever, areas of pulmonary crackles (rales), and silent zones in the lungs. Endoscopic examination showed no catarrhal secretions in the trachea, and no bronchoalveolar lavage was performed. The chronic pneumonia developed into neurological disorders, including incoordination, apathy, trembling, muscular weakness, and convulsions. Therapeutic treatment consisted of a 10-day course of corticosteroids, gentamicin, and streptomycin.

The animal was euthanized and a necropsy performed. Macroscopically, multiple soft gelatinous nodules were distributed randomly in the lungs, and the splenic white pulp was reactive. There was hyperemia and meningeal thickening in the CNS. Tissue samples of lungs, spleen, cerebral cortex, cerebellum, and spinal cord were submitted for virological and bacteriological examinations for differential diagnosis. Samples of lung, spleen, and CNS were fixed in 10% buffered formalin, embedded in paraffin, and 5-μm sections were stained with H & E stain for routine histology.

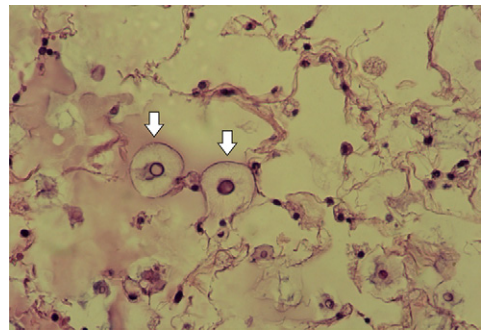

Fig. 1. *Cryptococcus* organisms (white arrows) stained by hematoxylin and eosin stain (H & E) in the meninges, surrounded by a thick clear mucoid capsule (halo) not stained by H & E (1000×).

Microbiological analyses of selected organs were negative for rabies, equine herpes viruses, equine encephalomyelitis, protozoal myeloencephalitis, and bacterial diseases.

In H & E sections, several cryptococcal organisms were observed in the meninges, enclosed within a thick clear mucoid capsule (halo) not stained by H & E (Fig. 1). The microscopic alterations observed at meninges of the spinal cord, cerebral cortex, and cerebellum were fibrin, edema, vascular congestion, and a moderate mononuclear inflammatory infiltrate characterized by lymphocytes and foamy cells (Fig. 2), as described by Zachary [3]. Differential staining was performed [3], and the presence of the thick mucoid capsule of several *C neoformans* confirmed by mucicarmine staining, and these organisms also stained with PAS and GMS (Easy Path kits).

The sections of the pulmonary parenchyma stained by H & E revealed small granulomas containing some *C neoformans* surrounded by lymphocytes, epithelioid cells, and a few giant multinucleated cells (Fig. 3). Areas of the pulmonary parenchyma contained cavities with an intense granulomatous reaction, where lymphocytes, macrophages, and *C neoformans* predominated. Some granulomas presented *C neoformans* in the central area, and the outer delineated layer of connective tissue was commonly infiltrated by lymphocytes and plasma cells. Histopathological

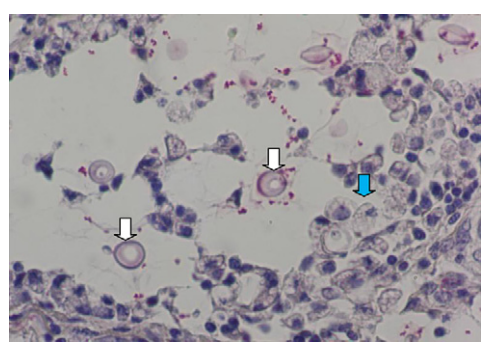

Fig. 2. Cryptococcal organisms in meninges. Only the thick mucoid capsule is stained by mucicarmine (white arrows). Several foamy cells and lymphocytes are visible (blue arrow) (1000×).

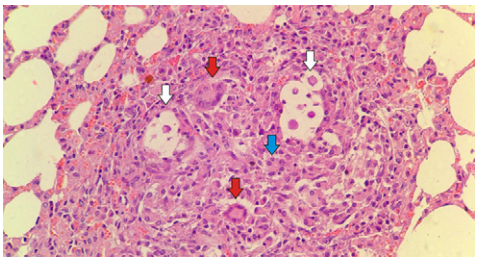

Fig. 3. Severe granulomatous inflammatory reaction, with *Cryptococcus neoformans* (white arrows) in the pulmonary parenchyma surrounded by granulomatous tissue, lymphocytes, epithelioid cells (blue arrow), and giant multinucleated cells (red arrow) (H & E stain) (400×).

lesions in the lungs were similar to those described by Kommers et al. [8] and López [21].

The macroscopic findings associated with histopathological lesions, and the special stains that identified the yeasts (PAS and GMS) and their thick mucoid capsule (mucicarmine), confirmed *C neoformans* as the cause of disease [3].

The diagnosis of cryptococcosis in the lungs and CNS suggests that, despite being uncommon in equines, this disease should always be considered in the differential diagnosis of equine pneumonia and encephalitis. In this case, the primary portal of entry was the lung, with hematogenous dissemination to the CNS causing meningitis, as described by other authors [3].

For animals with nonproductive and chronic pneumonia that is unresponsive to previous treatment, antemortem diagnosis by tracheal washing or bronchoalveolar lavage for microbiological analysis (bacterial, viral, and fungal culture) and cytologic examination have been recommended because they can reveal diseases difficult to diagnose, and identify infrequent causal agents such as *C neoformans*. A case report of seven horses with pulmonary cryptococcosis showed the importance of tracheal washes to identify the encapsulated yeast-like organisms in Wright's-stained sediment, but, because equine cryptococcosis carries a poor prognosis, treatment was not attempted in any of these animals, and they were euthanized [6]. In individuals with neurological symptoms, spinal fluid can be sampled to diagnose *C neoformans*, and the fungus can be visualized by using India ink, which outlines the organisms by negative contrast [22].

The epidemiological value of this case report lies in its demonstration that, in an equine race center, there is the risk of environmental contamination for other horses and human beings. Pigeons are natural reservoirs of *C neoformans* and pass large numbers of the organisms in their excrement [2]. Fundamental to preventing the spread of the pathogen is the adoption of stringent sanitary measures by eliminating birds from equine facilities, removal of feces, and disinfection of shelter locations [2,23]. The survival of the fungus can be influenced by several environmental factors, including humidity, temperature, sunlight, and biotic factors [23]. The fungus is able to survive in avian excreta for more than a year, and humidity can enhance its survival and proliferation in soil at lower temperatures (4°C to 26°C) but not at 37°C. Direct sunlight can significantly reduce its survival in soils and may account for the fact that pigeon excreta from outdoor sites are less heavily contaminated than from indoor sites [23]. In Brazil, the high humidity and warm temperatures of a tropical and subtropical climate at different seasons of the year allows the maintenance of fungus colonization in sites contaminated with pigeon excreta, making it difficult to control. Pigeons should be kept out of horse facilities to avoid environmental contamination.

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