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Case Report

Granulomatous meningoencephalitis due to *Halicephalobus gingivalis* in a horse

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Abstract

A 6-year-old, male, mixed-breed horse was presented for necropsy with history of blindness, ataxia, incoordination, peddling movements, nystagmus, depression, muscle spasms, abnormal appetite, mydriasis, abnormal behavior, and recumbency. There were no gross findings in the brain. Microscopically, there was meningoencephalitis characterized by a mild to moderate multifocal granulomatous inflammatory reaction, affecting mainly the cerebellum and, with lesser intensity, the thalamus and brain stem. Intralesional larval nematodes with morphology consistent with *Halicephalobus gingivalis* were observed. Based on the histopathological findings, a diagnosis of granulomatous meningoencephalitis by *H. gingivalis* was made.

Key Words: Horse diseases; *Halicephalobus gingivalis*; verminous meningoencephalitis; neuropathology.

Introduction

Halicephalobus gingivalis (formely *Micronema deletrix* or *H. deletrix*) is a free-living nematode commonly present in soil, manure and decaying organic matter, which can infect and cause disease in horses and humans. The genus *Halicephalobus* is in the order Rhabditida, superfamily Rhabditoidea, and family Rhabditidae. The parthenogenetic female is didelphic with reflexed ovaries. The pathogenesis, life-cycle, and route of this infection are poorly understood. It is presumed that infection occurs by contamination of mucosal, cutaneous, or ocular wounds by larvae in the environment, with hematogenous dissemination. The oronasal route of infection, causing tissue damage during its migration is also suspected and the parasite appears to establish its residence in the equine oral or nasal cavities (5). Pulmonary infection in two foals has lead to the suspicion that infection may occur by inhalation, transplacental, or transmammary routes (26).

H. gingivalis is a sporadic cause of granulomatous stomatitis, nephritis, and meningoencephalitis in horses. Although brain and kidney are the most commonly involved organs, nasal tissues, lymph nodes, lungs, spinal cord, adrenal glands heart, liver, stomach, bone, eye, and prepuce can also occasionally be affected (10, 11, 23, 26). One uncommon case of systemic infection was reported (24). Four human fatal cases of this parasitism have been reported (12, 19).

Neurologic disease associated to *H. gingivalis* has been observed in horses in many regions of the world, such as Central America (4), Japan (2, 25), USA (7, 10, 14, 15, 16, 23), Canada (6, 11), United Kingdom (3), Italy (8), Ireland (29), and Colombia (20) and Brazil (28) and there is a report of halicephalobiasis in zebras (15). In Brazil, this parasitism is restricted to one report (28) and in two large review studies that investigated neurologic diseases in horses in two wide separated regions of Brazil, no cases of *H. gingivalis* infection was diagnosed (21, 22). This

report documents an additional Brazilian case of meningoencephalitis by *H. gingivalis* in a horse occurring in the state of Goiás.

Case report

A 6-year-old, male, mixed-breed horse was submitted for necropsy after an afebrile disease with a clinical course of five days, with clinical signs including blindness, ataxia, incoordination, peddling movements, nystagmus, depression, muscle spasms, abnormal appetite, mydriasis, abnormal behavior, and recumbency, culminating with spontaneous death. The horse was from a small equine raising farm located in the county of Urutaí, southeast region of the state of Goiás, Brazil and occurred at the end of rainy season (April 2011). Only one out of a herd of 24 horses was affected. The horses graze *Brachiaria* spp., Tifton grass, and commercial ration. Oral ivermectin administration was done two months previously to clinical signs. Based in the information of the owner, neurological disorders never were observed in these horses of the farm. No gross changes were observed at necropsy.

Samples of cerebellum, thalamus, cervical spinal cord and telencephalic cortex of frontal lobes were collected and sent in refrigeration to immmunofluorescent antibody test which turned negative. Additional samples of liver, kidney and the remaining brain were collected and fixed in 10% buffered formalin and processed routinely for histopathological evaluation and stained with hematoxylin and eosin.

Microscopically, the brain lesions were observed mainly in the cerebellum and with lesser intensity in midbrain, thalamus and pons. Mild to moderate, multifocal perivascular infiltration of lymphocytes, plasma cells, foamy macrophages (*gitter cells*), and occasional multinucleated giant cells were seen in the leptomeninges, neuropil and white matter (Fig. 1). In addition, rhabditiform larval nematodes with pointed tail and smooth thin cuticle were also observed in the Virchow-Robin spaces, usually surrounded by macrophages, lymphocytes, and plasma cells (Fig. 2). The parasites are 14-18 μm in diameter and 80-110 μm in length, had low, indistinct platymyarian-meromyarian musculature, and numerous deeply basophilic 1-3 μm internal structures. Numerous axonal spheroids were identified adjacent to areas of perivascular inflammation. There were no microscopic changes in the liver and kidney.

Based on the histopathological findings, a diagnosis of granulomatous meningoencephalitis associated with *H. gingivalis* infection was made.

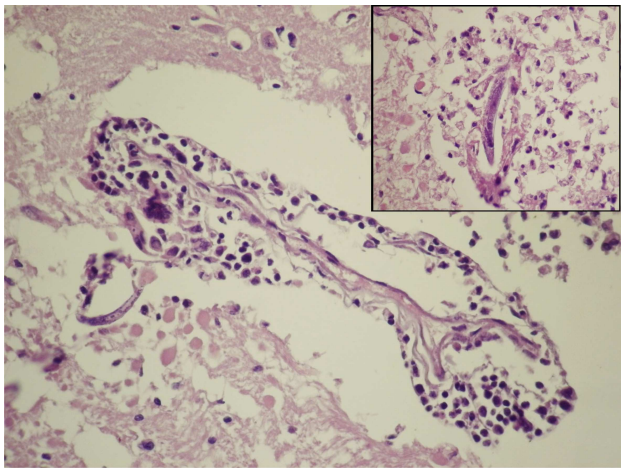

Figure 1 - Midbrain, horse. Granulomatous meningoencephalitis by *Halicephalobus gingivalis*. Inflammatory infiltrate of lymphocytes, plasma cells, foamy macrophages (*gitter cells*), and multinucleated giant cells adjacent to *H. gingivalis* larva in the perivascular space. Hematoxylin and eosin, 20X.

Inset: Brain stem, pons, horse. Granulomatous meningoencephalitis by *Halicephalobus gingivalis*. Rhabditiform larval nematode with pointed tail and smooth thin cuticle surrounded by many foamy macrophages in the perivascular space. Hematoxylin and eosin, 20X.

Discussion

Usually, infection by *H. gingivalis* affects adult 5-24 year-old horses (1, 2, 6, 10, 11, 16, 18, 23, 24, 25, 28) although a single exceptional case of halicephalobiasis is reported in 13-week-old foal (7). The case of this report was in the lower end of the usually describe age spectrum for this condition.

In the present case, the clinical signs and mainly the histopathology were paramount for the diagnosis of granulomatous meningoencephalitis by *H. gingivalis.* Cerebrospinal fluid (CSF) examination can be a complementary tool for the diagnosis of nematodiasis affecting the central nervous system (CNS) of horses, but the CSF changes are not specific (9). When *H. gingivalis* parasitizes urinary or reproductive tracts, urine and semen samples can be useful for the clinical diagnosis (14). In these cases, the urogenital route of infection has been suggested (14). In the present case, there were no signs of wounds, trauma, or lacerations in the mouth and adjacent tissues or skin that could elucidate the port of entry of infective larvae. As was noted in other reported cases (7, 15, 23) gross lesions were not observed in the horse of the current report, however, grossly visible edematous leptomeninges have been reported in the meningoencephalitis by *H. gingivalis* (25).

The brain microscopic findings detected in this study were similar to those observed in other investigations (6, 23, 25). Histologically, the larval nematodes were observed free in the Virchow-Robin spaces surround by inflammatory cells. This finding is compatible with the

proposed hematogenous route of dissemination (31). In some reports, the nematodes are present in the walls of affected brain arteries (6). Interestingly, eosinophils are usually meager in the inflammatory infiltrate (1, 7) or absent (2) in the cellular exsudato, as was the case of the current report. Some authors suggest that lesions rich in eosinophils may represent reinfection (10), whereas few or absent eosinophils would be indicative of primary infection (6). In the present case, inflammatory response was only composed of mononuclear cells.

The differential diagnosis includes other free-living rhabditoid parasites that affect horses, such as *Pelodera strongyloides*, *Strongyloides westerii*, and *Cephalobus* sp. Differentiation is based upon location and severity of lesions and parasite morphology. All these nematodes have a rhabditiform esophagus; however, only *Halicephalobus* has a reflexed ovary and a pointed tail. *Cephalobus* sp. can be distinguished from *H. gingivalis* by its blunt posterior end and differences in the shape of the stoma and esophagus (13). *Cephalobus* was reported in the mammary gland of a mare (13). *P. strongyloides* causes a self-limiting dermatitis normally confined to the ventral abdomen and limbs. In addition, *P. strongyloides* have two lateral alae, as well as two lateral cords noted by two dense nuclei. The life-cycle of *S. westerii* involves cutaneous penetration by larvae; adults and eggs are not found in the skin. *S. westerii* have alae, which are absent in *Halicephalobus* spp. (13). Other nematodes, such as *Strongylus vulgaris*, *Draschia megastoma*, *Angiostrongylus cantonensis*, or protostrongylides can migrate erratically through the CNS of horses and cause clinical signs and morphologic lesions similar to those in *H. gingivalis* (17, 27, 30). Other important neurologic disorders of horses must be included in the differential diagnosis, such as leucoencephalomalacia, viral encephalitis, equine mieloencephalitis by *Sarcocystis neurona* and *S. hughesi*, hepatic encephalopathy, and trypanosomiasis by *Trypanosoma evansi*.

Further epidemiologic and pathological studies are necessary to determinate the specific route of infection, pathogenesis and prevalence of the halicephalobiasis in Goiás State, Brazil. To the authors knowledge, this is the first case of *H. gingivalis* infection in horse diagnosed in Midwest Brazil.

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