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PATHOLOGY

Cerebral infarction and thrombotic meningoencephalitis in a calf due to candidiasis

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ABSTRACT: We described a case of cerebral infarction and thrombotic meningoencephalitis due to candidiasis in a seven-month-old calf. The death occurred three days after the onset of apathy, fever, and the head's lateral deviation to the left. Macroscopic changes in the brain consisted of asymmetry of telencephalic hemispheres; the right hemisphere was enlarged, causing cerebellar herniation. A focally extensive red area was observed on the surface of the right occipital lobe. At cross-sections of the fixed brain, the lesions revealed to be extensive, red-brown, soft or cavitated areas affecting the white and grey matter from the level of the thalamus to the cerebellum and compressing subjacent structures. Histologically, there was acute, coalescent, multifocal necrosupurative meningoencephalitis, associated with vasculitis, congestion, thrombosis, edema, infarction, and intralesional fungal hyphae. The diagnosis of cerebral infarction and thrombotic meningoencephalitis due to candidiasis was made by the pathological changes, the staining and morphological characteristics of the agent, and immunohistochemistry. The cerebral infarction and thrombotic meningoencephalitis in cattle can result from vascular lesions due to infection by *Candida* sp.; although uncommon, this case demonstrated that candidiasis should be part of a list of differential diagnoses of severe brain injuries in cattle.
Key words: diseases of cattle, *Candida* sp., neuropathology, infarction, vascular lesions.

Infarto cerebral e meningoencefalite trombótica em um bezerro devido a candidíase

RESUMO: Descreve-se um caso de infarto cerebral e meningoencefalite trombótica devido a candidíase em um bezerro de sete meses de idade. A morte ocorreu três dias após o início de apatia, febre e desvio lateral da cabeça para a esquerda. As alterações macroscópicas no cérebro consistiam em assimetria dos hemisférios telencefálicos; o hemisfério direito estava aumentado, causando herniação cerebelar. Uma extensa área vermelha focal foi observada na superfície do lobo occipital direito. Nos cortes transversais do encéfalo fixado, as lesões revelaram áreas extensas, marrom-avermelhadas, moles ou cavitadas, afetando a substância branca e cinzenta desde o nível do tálamo até o cerebelo e comprimindo as estruturas subjacentes. Histologicamente, havia meningoencefalite necrossupurativa multifocal aguda, coalescente, associada a vasculite, congestão, trombose, edema, infarto e hifas fúngicas intralesionais. O diagnóstico de infarto cerebral e meningoencefalite trombótica devido a candidíase foi feito pelas alterações patológicas, coloração e características morfológicas do agente e imuno-histoquímica. O infarto cerebral e meningoencefalite trombótica em bovinos pode resultar de lesões vasculares devido à infecção por *Candida* sp.; embora incomum, este caso demonstra que a candidíase deve fazer parte de uma lista de diagnósticos diferenciais de lesões cerebrais graves em bovinos.
Palavras-chave: doença de bovinos, *Candida* sp., neuropatologia, infarto, lesões vasculares.

INTRODUCTION

Infarction refers to a local area of necrosis secondary to peracute ischemia, usually resulting from vascular blockage due to thrombosis or thromboembolism (MOSIER, 2017). Lesions leading to occlusion of vessels in the central nervous system (CNS) are reportedly uncommon in domestic animals (ECCO et al., 2016). However, infection by several pathogens are the cause of severe inflammatory lesions in the brain of cattle (CANTILE & YOUSSEF, 2016; MILLER & ZACHARY, 2017; BIANCHI et al., 2020) including fungal agents such as *Cryptococcus* sp. (RIET-CORREA et al., 2011), *Zygomycetes* (KONRADT et al., 2017; BIANCHI et al., 2020) and *Candida* sp. (VILANDER et al., 2016).

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In cattle, *Candida* spp. infections are usually reported in cases of mastitis (SANTOS & MARIN, 2005), placentitis and abortion (FOLEY & SCHLAFER, 1987), and gastric lesions with diarrhea (WADA et al., 1994). Infections that result in lesions of the CNS are more frequently described in humans (PAPPAS et al., 2009) and less commonly in companion animals (ALVES et al., 2020), and rarely in cattle, in which species there is only one reported case of brain abscess by *Candida etchellsii* in an aborted calf, that until then was not considered a pathogenic microorganism (VILANDER et al., 2016). We here reported a case of cerebral infarction and thrombotic meningoencephalitis due to candidiasis in a calf from the semiarid region of the state of Paraiba, Northeastern Brazil.

MATERIALS AND METHODS

A calf with a history of emaciation, apathy, dyspnea, fever, and lateral deviation of the head to the right died and was submitted to necropsy. Samples of the organs of the thoracic and abdominal cavities, and central nervous system were fixed in 10% buffered formalin, processed routinely, embedded in paraffin wax and cut into 3 µm sections. The sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Grocott's methenamine silver stain (GMS).

An immunohistochemistry (IHC) protocol was performed using a polyclonal antibody made in rabbit anti-*Candida albicans* (Abcam, ab53891), diluted 1:100, followed by polymer HiDef-HRP (Cell Marque; Millipore Sigma), and chromogen 3,3' diaminobenzidine (DAB; Kit EasyLink One [EasyPath]). As positive control, histological sections from a confirmed case of candidiasis by *Candida albicans* in dog by mycological culture and molecular test was used. Sections from the same dog and tested calf were used as negative controls, with the primary antibody replaced by phosphate buffered saline containing 0.5% polysorbate 20 (Tween 20).

RESULTS

A seven-month-old female Swiss Brown calf presented emaciation, apathy, dyspnea, fever, rough hair coat, congested ocular mucosa, and lateral deviation of the head to the right. It died within three days of the onset of the clinical signs. This calf was the only one animal affected in its herd.

At necropsy, the right cerebral hemisphere was enlarged, and there was cerebellar slight herniation through the foramen magnum. A large focally extensive softened focal red area was seen over the surface of the occipital lobe (congestion and haemorrhage of leptomeningeal vessels). Although smaller, similar areas were observed over the cortical and parietal surfaces of temporal and parietal lobes. The borders of these areas were irregular (Figure 1A). In cross-sections of the formalin-fixed brain, the lesions appeared as large softened or cavitated red-brown areas involving the grey and white matter and hippocampus with compressing the thalamus, third ventricle and the colliculi (Figure 1B).

Microscopically, brain lesions included severe subacute, extensive necrosupurative meningoencephalitis, associated with vasculitis, congestion, thrombosis, edema, infarction, and intralesional fungal hyphae. The leptomeninges were markedly thickened by edema and abundant, mostly degenerated, neutrophils, epithelioid macrophages, and rare lymphocytes, associated with fibrin and hemorrhages (Figure 2A). The infiltrate was mainly distributed around blood vessels and spread multifocally to the adjacent grey matter. There were also vasculitis, fibrinoid necrosis, thrombosis, and congestion in the leptomeninges' blood vessels and adjacent neuropile. Extensive infarction areas were in the neuropile, characterized by dilation and vacuolization of the perineural and perivascular spaces associated with neuronal necrosis, rarefaction of neuropile, hemorrhage, edema, infiltration of macrophages with foamy cytoplasm (gitter cells), and myriads of intralesional fungal organisms. Clusters of fungal hyphae were seen free in the infarcted neuropile areas or in the leptomeninges, within thrombi, or walls of blood vessels. No lesions were observed in other organs.

The hyphae have basophilic walls in the HE-stained sections appearing as tubular negative images in longitudinal and transverse sections. They stained by PAS (Figure 2B) and GMS (Figure 2C) techniques. Morphologically, the hyphae had parallel walls, rarely septate with angular ramification, and measuring 4 to 15 µm a diameter. The IHC with the polyclonal anti-*Candida albicans* antibody revealed strong immunolabelling of the cytoplasm and wall of hyphae in brown (Figure 2D).

DISCUSSION

The diagnosis of cerebral infarction and thrombotic meningoencephalitis due to candidiasis was based on the microscopic lesions, and confirmed by immunohistochemistry. Several infectious

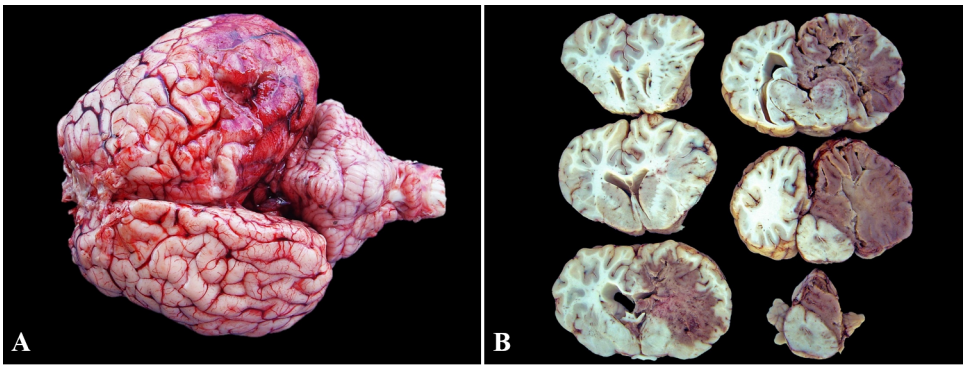

Figure 1 - Cerebral infarction and thrombotic meningoencephalitis in a calf due to candidiasis. A) Brain. Asymmetry of the right cerebral hemisphere. There is a focal softened reddish area in the right occipital cortex and slight cerebellar herniation. B) Cross-sections of the formalin-fixed brain. Grey and white matter in the right occipital and temporal lobes and hippocampus are diffusely brown, cavitated, and compressing the thalamus and colliculi.

microorganisms can cause meningoencephalitis associated with vascular damage in cattle, including infections by *Mortierella wolfii*, *Streptococcus*, *Chlamydophila pecorum*, *Escherichia coli*, *Histophilus somni* and *Macavirus* (CANTILE & YOUSSEF, 2016; CURTIS et al., 2017; KONRADT et al., 2017; MILLER & ZACHARY, 2017; BIANCHI et al., 2020).

The sequelae of vascular obstructions depend on the obstructed vessel's type and size, degree and duration of ischemia, and tissue vulnerability to anoxia (CANTILE & YOUSSEF, 2016). However, when the arterial blockage is complete, it usually results in necrosis of the supplied area (MOSIER, 2017). In this case, vascular lesions induced by the fungus, such as vasculitis and thrombosis, probably obstructed the middle and caudal cerebral arteries or one of its main branches, responsible for the vascular supply of the caudal half of the brain, resulting in immediate and prolonged ischemia with infarction of the brain.

The yeasts of the *Candida* genus reside as commensal microorganisms in the mucous membranes and on the skin (GIUFFRIDA, 2016). Thus, various tissues can be affected depending on the site of infection, being the skin and organs of the digestive system, the most affected ones. In the cutaneous infections exudative, pustular to ulcerative lesions are observed (HARGIS & MAYERS, 2017). In the gastrointestinal tract, lesions are described in the tongue, esophagus, stomach, rumen, abomasum and intestine, characterized by ulcerated areas or nodules covered by yellowish, lumpy or serosanguineous material (GALIZA et al., 2014). However, in our case, it was not possible to determine the portal of entry of the infection, but the agent is believed to have penetrated the mucosal or skin barriers, subsequently with invasion of blood vessels and dissemination to the CNS.

There are rare reports of infections by *Candida* sp. in animals that describe vascular lesions caused by the agent (ALVES et al., 2020), as seen in the present case. In humans with cerebral candidiasis, the vascular complications described include cerebral infarction, mycotic aneurysms, and subarachnoid hemorrhage (LIPTON et al., 1984).

Candida species are known as opportunistic pathogens and need a compromise in the body defense mechanisms to establish clinical disease (GIUFFRIDA, 2016). The determining factors for candidiasis in cattle include intense and prolonged antibiotic therapy (MILLS & HIRTH, 1967), nutritional deprivations, and immune compromising diseases (GIUFFRIDA, 2016), including bovine viral diarrhea virus infection (VILANDER et al., 2016). However, in this case, there was no primary condition that could trigger an immunosuppression condition. Some cases have been described without characteristic signs of immunosuppression (WILLEMS et al., 2017).

In our case, the clinical signs and the clinical course are similar to other neurological conditions of cattle, including brain abscesses, trauma, and cerebrocortical necrosis. Thus, the necropsy,

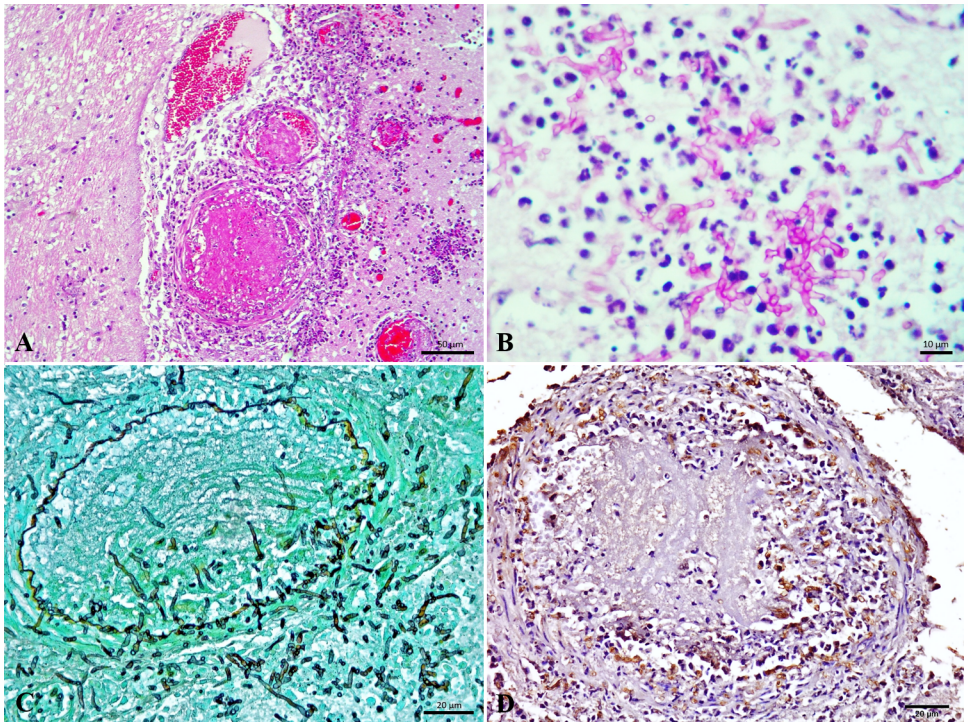

Figure 2 - Cerebral infarction and thrombotic meningoencephalitis in a calf due to candidiasis. A) Brain. Multiple blood vessels with vasculitis and thrombosis are surrounded by a cellular infiltrate consisting of neutrophils and epithelioid macrophages in the leptomeninges and adjacent neuropile. [Hematoxylin and eosin. Bar, 50 µm]. B) A cluster of hyphae surrounded by neutrophilic infiltrate. [periodic acid-Schiff. Bar, 10 µm]. C) Numerous hyphae in the neuropile and vascular wall. [Grocott's methenamine silver stain. Bar, 20 µm]. D) Vascular thrombosis. Strong immunolabelling of the hyphae (in brown) for polyclonal anti-*Candida albicans* antibody. [Immunohistochemistry, 3,3' diaminobenzidine. Bar, 20 µm].

followed by histologic study and IHC, was necessary for the diagnosis. In addition, the agent's culture and molecular examinations are often used to confirm the agent (VILANDER et al., 2016). However, the nonspecific gross lesions made it difficult to suspect of fungal infection, making it impossible to collect tissue samples for other specific complementary diagnostic methods. In humans, cerebral candidiasis is rarely diagnosed clinically due to the lack of characteristic clinical manifestations, making the diagnosis only at postmortem examination the rule in a large percentage of the cases (SÁNCHEZ-PORTOCARRERO et al., 2000).

CONCLUSION

We concluded that cerebral infarction and thrombotic meningoencephalitis in cattle can result from vascular lesions due to infection by *Candida* sp.; although uncommon, this case demonstrated that candidiasis should be part of a list of differential diagnoses of severe brain injuries in cattle.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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