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Malignant catarrhal fever-like lesions associated with ovine herpesvirus-2 infection in young calves (*Bos indicus*): a case report

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ABSTRACT: Infection of susceptible ruminants, including domestic cattle (*Bos taurus*) and American bison (*Bison bison*), with ovine herpesvirus-2 (OvHV-2) may provoke the fatal vasculitis and lymphoproliferative syndrome, known as malignant catarrhal fever (MCF), reported worldwide. To the best of our knowledge, this is the first report of a clinical case of MCF-like lesions associated with ovine herpesvirus-2 (OvHV-2) infection in young calves (*Bos indicus*) including central nervous symptoms that occurred in Três Lagoas city, Mato Grosso do Sul state, a border town near São Paulo state, Brazil. The diagnosis was based on typical histological lesions characterized by systemic lymphohistiocytic and fibrinoid vasculitis, confirmed by polymerase chain reaction and subsequent phylogenetic analysis of detected OvHV-2 sequences. This finding indicates that MCF disease is spread among herds concentrated in border areas between Mato Grosso do Sul and São Paulo states.

KEY WORDS: ovine herpesvirus-2, malignant catarrhal fever (MCF), meningoencephalitis, cattle.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Malignant catarrhal fever (MCF), a fatal lymphoproliferative disease of ruminant species including domestic cattle and wild living ruminants, is caused by gammaherpesvirus usually associated with malignant catarrhal fever viruses (1). As to its epidemiology, many reports have described this virus's worldwide distribution, but so far its disease status has not been completely documented in Brazil (2-5). Among bovine encephalitis disorders, rabies virus infection is the most controlled infectious disease with a national sanitary program ongoing in our country (6). Secondly, the bovine herpesviruses type 5 (BoHV-5) and type 1 (BoHV-1) have assumed a special role in revealing the etiology of neurological diseases in Latin America, based on the negative results of rabies investigations (7, 8). In this sense, undefined diagnosis leaves to farmers the responsibility of taking informal sanitary measures. Besides, due to significant losses incurred by the cattle industry as a result of livestock neurological disorders, the USA and the European Union have imposed strict sanitary controls that directly affect Brazilian farmers, who are considered an important source of meat to Europe and Asia.

Studies of MCF pathogenesis have demonstrated that the American bison (*Bison bison*), Bali cattle (*Bos javanicus*) and many species of deer (*Cervidae*) are highly susceptible to the disease, while cattle are relatively resistant to OvHV-2-induced MCF. However, high morbidity rates of MCF infection in cattle have been reported worldwide (9, 10). Herein, we describe MCF-like lesions in young calves (*Bos indicus*) suspected to be infected with OvHV-2 virus, confirmed by polymerase chain reaction (PCR) and nucleic acid sequencing among affected Brazilian herds.

CASE REPORT

The outbreak occurred from January to March, 2008, in an extensive beef cattle herd in Três Lagoas, a border city in Mato Grosso state near northern São Paulo state, Brazil. Mixes of sheep and cattle of different ages have been frequently observed. The herd consisted of 1000 cross-bred Nelore, among which 12-month-old steers were affected. Clinical signs included ataxia, tonic-clonic spasm, disorientation and tremor of the head. The animals died 24 hours after the first manifestation of infection. One live animal was brought to the Pathology Section of the School of Veterinary Medicine for analysis. Samples from all described organs were submitted to PCR, targeting the glycoprotein B region from OvHV-2 as previously described

(11). The two primers used, GlycoF02 and GlycoR01, were able to amplify a product of 424bp. The DNA extraction and the PCR condition were performed according to a previous description (12). No positive control was used, although the negative results were based on normal brain obtained from a slaughterhouse. Unstained sections (4 μm) were used for the direct immunohistochemical examination after deparaffinization, rehydratation and washings in buffered saline added to 0.1% Tween 80. The first step was to microwave the sections in citrate buffer (pH 6.1) for 15 minutes at 700 W to activate the viral antigen, normally damaged by formaldehyde fixation. Just before staining, slides were treated three times with hydrogen peroxide 50% (30 V) for 30 minutes to inactivate endogenous peroxidase, commonly found in inflammatory reactions. So, the slides were placed in buffered saline for ten minutes five consecutive times, to remove the residues in each interval between steps of the reaction whereas the non-specific bindings were blocked using dried 15% nonfat milk to decrease the background activity for 90 minutes. The antigen was revealed by the avidin-biotin complex (ABC) immunoperoxidase method to search for the bovine spongiform encephalopathy (BSE) with some modifications (13). The optimal monoclonal antibody (rat anti-GFAP, Zymed®, Invitrogen, USA) dilution was 1:400 accomplished in PBS plus 10% nonfat dried milk; slides were covered by 200 μL of diluted antibody overnight at 4°C in a humidified chamber. After five washings, the 100 μL/slide of ABD complex (DakoCytomation, USA) was added to each slide and incubated 1 h at 37°C. In addition, substrate made fresh in the dark, by mixing equal volumes of 0.02% hydrogen peroxide and 0.6 mg DAB (3,3´-diaminobenzidine tetrahydrochloride, Gibco BRL, USA), was added to the slides for 30 minutes at room temperature. The reaction was stopped by washing with tap water and the specific brown color was revealed after counterstaining with Meyer's hematoxylin for two minutes. An intense dark red deposit indicated a positive result whereas the negative controls consisted of sections treated with buffered saline instead of monoclonal antibody.

An outbreak of an unknown disease affecting calves (*Bos indicus*) – that presented fever and central nervous disorders including ataxia, tonic-clonic spasm, disorientation and tremor of the head – was studied. The 1-year-old animals, from São Paulo state, Brazil, were submitted to necropsy. Clinically, the animals showed central nervous disorders, elevated body temperatures, enlarged lymph nodes and

absence of diarrhea. Similar signs have been documented in *Bos taurus* worldwide. Figure 1 – A displays the most characteristic lesion, namely a cloudy cornea, observed in all animals.

The enlargement of lymph nodes, spleen and liver, as well as liver hemorrhage, pulmonary congestion and tiny white spots distributed multifocally in the kidneys (Figure 1 –B and C) could be observed. In addition, the animals presented erosive and ulcerative lesions in esophagus and abomasums (Figure 1 – D).

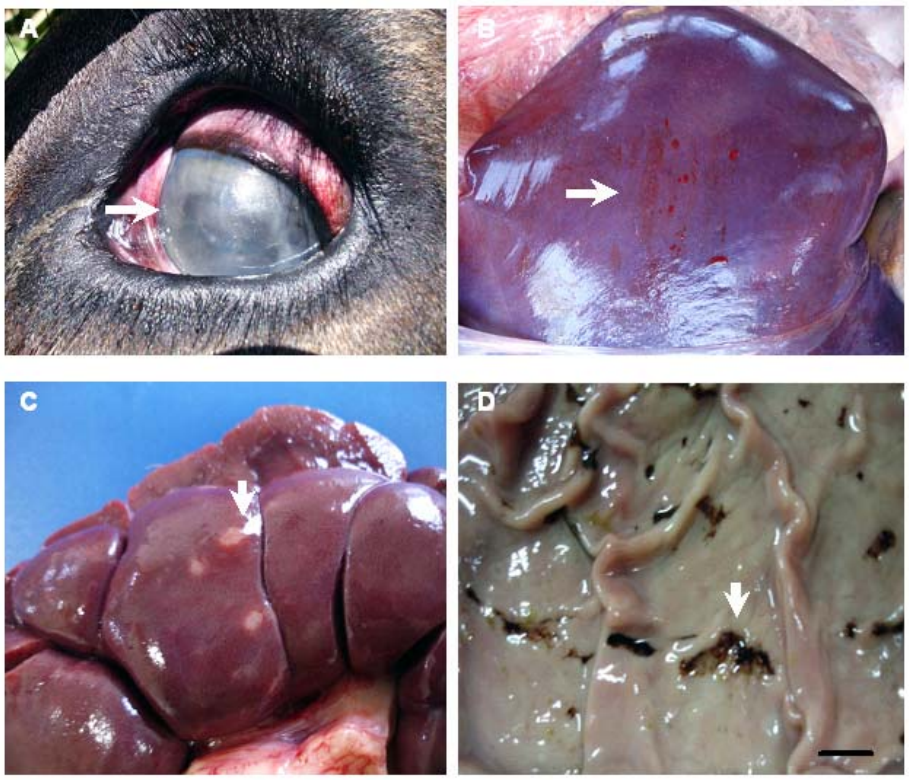
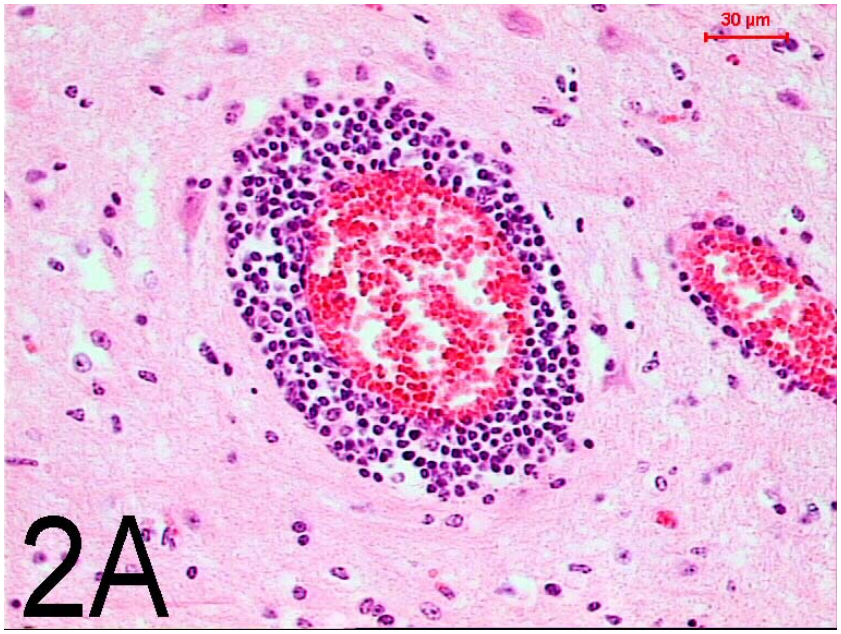


Figure 1. Gross lesions observed in young calf with suspected acute malignant catarrhal fever infection. (**A**) The most common lesion, characterized by a cloudy cornea, observed in all animals (arrow). (**B** and **C**) Liver hemorrhage and tiny white spots distributed multifocally in a kidney (arrows). (**D**) Abomasum mucosal surface displaying necrosuppurative ulcerations (arrows) with yellow-green exudates. Bar 2 cm.

Confirmatory diagnosis was based on the histological demonstration of a generalized lymphohistiocytic vasculitis with fibrinoid necrosis in multiple organs, including lymph nodes, liver, kidneys, adrenal gland and brain (Figure 2 – A). These findings corroborate those obtained by experimental infection performed in cows which

strongly suggest virus replication (10). Astroglial cells were the most abundant cell type in the infected brain; however, our knowledge about their function in MCF disease remains limited (14). In fact, astrocytes are suspected of being involved in a wide range of neuropathologies associated with the degeneration process. In this sense, astrocytes express the intermediate filaments called glial fibrillary acidic proteins (GFAP), which were first isolated from brain lesions of patients with multiple sclerosis (15). As shown by immunohistochemistry, the majority of glial cells were surrounding the vasculitis areas that were expressing GFAP (Figure 2 – B). The emerging picture is highly interesting and it suggests that GFAP could be a structure of great importance in the neuropathogenesis of MCF lesions, a phenomenon that should be investigated in future experiments.



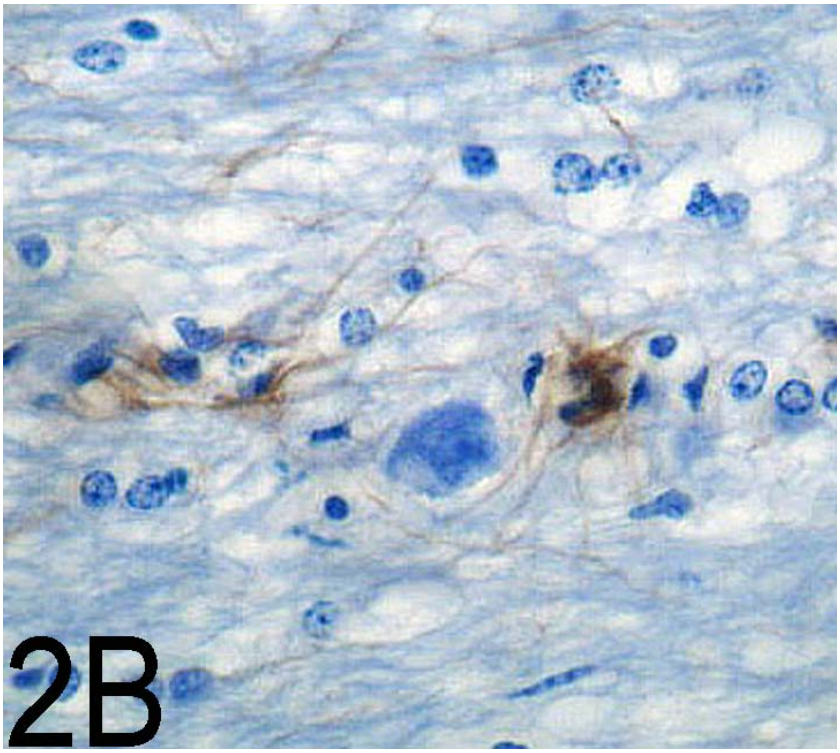


Figure 2. (**A**) Histological lesions characterized as vasculitis, predominantly perivascular lymphocytic inflammation in the brain and (**B**) immunohistochemical findings of glial fibrillary acidic protein (GFAP) expression around brain vasculitis areas. Bar 30 µm.

Since members of the gammaherpesvirinae in general cannot be propagated easily on cell culture, a molecular approach represents the method of choice to verify histopathological findings (10, 12). Samples from different organs − namely the brain, mesenteric lymph node and kidneys − were subjected to the polymerase chain reaction (11). Primers designed for the glycoprotein B region of OvHV-2 were applied and a 424-bp fragment was amplified. Upon gel electrophoresis all samples showed DNA bands at the expected sizes. Comparison of nucleotide sequences of the glycoprotein B region to other herpesvirus samples retrieved from the gene bank revealed similarities of 99.05-99.78% for OvHV-2 (results not shown).

Furthermore, gammaherpesviruses of veterinary interest cover a surprisingly wide range of hosts, both on the cellular level and in relation to susceptible animals. In addition, it should be emphasized that the lytic cycle was crucial in the acute death of affected animals, in spite of the many studies that characterize lytic cycles in gammaherpesvirus cases as rare (12). Usually, latent infection in cattle can lead to clinical disease even in the absence of further sheep contact, although in cattle,

provoking the disease via the reactivation of the virus is uncommon. The present study demonstrated MCF-like lesions documented in young calves in Brazil. Additionally, this study showed that astrocyte intermediate filaments found around vasculitis areas in brain sections may constitute an important key to elucidating its neuropathogenesis. However, further studies are necessary to determine the epidemiology of virus distribution among sheep and cattle. Herein, the clinical signs observed in young beef calves − which led to virus reactivation that, in turn, caused MCF-like lesions − prove that some of the cattle might have already been infected with the virus.

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