Non-suppurative Myocarditis Associated with Bovine Viral Diarrhea Virus Infection in Calves in the State of Mato Grosso do Sul, Brazil

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ABSTRACT

Background: Bovine viral diarrhea virus (BVDV) refers to a heterogeneous group of viruses belonging to the family Flaviviridae and genus Pestivirus. This family of viruses is one of the main pathogens of cattle and causes significant economic losses to the cattle industry worldwide. BVDV is an enveloped virus with a diameter of 45 nm and single-stranded RNA genome of 12.5 kb. BVDV infection has been associated with a number of clinical manifestations ranging from unapparent infection and mild signs to acute illness and death. In general, calves are more susceptible to BVDV infection, but adult cattle can develop the clinical disease if they are infected with highly virulent virus strains. This study describes clinical, anatomopathological and epidemiological findings of a BVDV outbreak in calves in the state of Mato Grosso do Sul, Brazil.

Materials, Methods & Results: The outbreak occurred in the town of Agua Clara in the state of Mato Grosso do Sul. Epidemiological and clinical data were collected by the farm manager during a visit to the property. The outbreak involved two Nelore heifer calves that died between 30 and 40 days of age. One calf was taken to the Laboratory of Pathological Anatomy (LAP) of the Faculty of Veterinary and Animal Husbandry, Federal University of Mato Grosso do Sul (FAMEZ/UFMS). The calf was necropsied, and white streaks were seen on the heart, indicating congestive failure with swelling of body cavities and congestive hepatopathy (nutmeg liver). Fragments of different organs and tissues were collected during necropsy, fixed in 10% formalin for 48 h, embedded in paraffin, cut in 5 µm sections and stained with hematoxylin-eosin (HE) or analyzed by immunohistochemistry (IHC) in the Veterinary Pathology sector of the Federal University of Rio Grande do Sul (UFRGS). Histologically, the heart lesion was characterized by fibrous coagulative necrosis associated with marked infiltrate (predominantly lymphocytic) and some macrophages. The diagnosis was confirmed by immunohistochemical agent identification in Peyer’s patches within the intestine.

Discussion: The diagnosis of congestive heart failure due to myocarditis caused by BVDV infection was confirmed by the IHC technique. While in other countries, myocarditis caused by natural infection in cattle and experimental infection in goats and sheep due to BVDV has been described, there have been no reports of this clinical and pathological manifestation of the disease in Brazil. The heart lesions observed in the outbreak should be differentiated from similar injuries caused by certain plants and from Neospora caninum infection. In the present study, while the virus was identified by immunohistochemistry only in Peyer’s patches, BVDV was considered to be the cause of the cardiac lesions by a process of elimination and because there is no correlation between the amount of viral antigen and the location of histological lesions. Other studies have used the IHC technique to detect BVDV antigen in other tissues of cattle and observed that the antigen is not uniformly distributed among the organs, suggesting that no specific organ of aborted fetuses can be chosen for BVDV diagnosis. Immunohistochemistry was shown to be an efficient method for detecting the antigen in the Peyer’s patches of infected calves. This is the first report of non-suppurative myocarditis associated with BVDV causing perinatal calf death with agent identification in Mato Grosso do Sul. However, these data are insufficient to determine the importance of BVDV infection in terms of reproductive losses in this state because the methodological approaches used were different from those adopted in earlier studies.

Keywords: bovine viral diarrhea virus, myocarditis, immunohistochemistry, diagnosis.
INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an enveloped virus that can be classified into two biotypes, cytopathic (CP) and non-cytopathic (NCP), depending on their effect in cell culture. Because of its high antigenic variability, the virus can also be sub-divided into two major antigenic groups: type 1 and type 2 [37].

The clinical manifestations of BVDV infection include respiratory disease, acute or chronic gastrointestinal syndrome, hemorrhagic syndrome with thrombocytopenia, mucosal disease, skin diseases and immunodepression [10,16,19,22]. The infection of pregnant females produces a series of reproductive failures such as embryo resorption, abortion, mumification, fetal malformations, stillbirth, and the birth of weak and unviable calves [18]. Hypomyelination is less frequent [33], but immunotolerant and persistently infected (PI) calves are commonly born when the infection occurs between 60 and 90 days of pregnancy [2]. Congenital defects seen in fetuses include cerebellar hypoplasia, microencephaly, hydrocephalus, hydranencephaly, porencephaly, hypomyelination, osteochondrosis, growth delay, optic neuritis, retinal degeneration, thymic hypoplasia, hypothyroidism, alopecia, osteosclerosis, microphthalmia, cataracts and overbite [7]. Necrotizing inflammatory lesions in the fetal myocardium, lung and eyelid, which are associated with abortion, may also occur [34]. In sheep and goats, BVDV infection also causes lymphocytic myocarditis associated with disseminated multifocal necrosis [7,26].

The present study aimed to describe the epidemiology, clinical signs, pathology and laboratory diagnosis of an outbreak of BVDV with calf mortality in Mato Grosso do Sul (MS), Brazil.

MATERIALS AND METHODS

Epidemiological and clinical data on the outbreak were collected. Information on the outbreak was provided by the farm manager during a visit to the property when the pasture was inspected to search for cardiotoxic plants such as *Tetrapterys multiglandulosa*. One affected calf was taken to the Laboratory of Pathological Anatomy (LAP) of the Faculty of Veterinary and Animal Husbandry, Federal University of Mato Grosso do Sul (FAMEZ/UFMS). The calf was necropsied, and tissues were collected for laboratory diagnosis.

Fragments of different organs and tissues (brain, tongue, lung, heart, liver, spleen, intestine and kidneys) were collected during necropsy and fixed in 10% formalin for 48 h. Following routine procedures, the fragments were embedded in paraffin and cut into five-micrometer sections. These sections were stained with hematoxylin-eosin (HE) or analyzed by immunohistochemistry (IHC) in the Veterinary Pathology sector of the Federal University of Rio Grande do Sul (UFRGS).

IHC was performed using the commercial DAKO kit¹ according to the technique described by Haines et al. [23]. The kit contains a secondary antibody and avidin complex conjugated to peroxidase in addition to the commercial monoclonal anti-BVDV 15C5 antibody², which was diluted at 1:1500 in phosphate buffer solution.

RESULTS

The outbreak involved two Nellore heifer calves that died between 30 and 40 days of age. They belonged to a herd of 4300 animals, including 900 calves divided into lots of 100 animals. The morbidity and mortality rates were 0.0005% and 100%, respectively. The cattle were vaccinated against FMD, brucellosis, leptospirosis, blackleg and botulism. *Tetrapterys multiglandulosa* or other plants responsible for cardiomyopathies were not found on the premises. According to the owner, no cattle had been introduced on the property in the three years preceding the onset of the outbreak.

The analyzed calf died 10 min after experiencing ataxia, falling and paddling. She had exhibited good body condition. At necropsy, a reddish liquid was found in the thoracic (100 mL) and abdominal (200 mL) cavities and in the pericardial sac (20 mL). In the heart, whitish streaks were diffusely distributed throughout the myocardium (Figure 1). The omentum was thickened, yellowish and gelatinous (edema). The liver was enlarged, displaying rounded edges and interspersed with dark and light areas (nutmeg liver). The liver was enlarged, displaying rounded edges and interspersed with dark and light areas (nutmeg liver).

The histological examination showed extensive areas of coagulative necrosis of myocardial fibers associated with a marked, predominantly lymphocytic infiltrate and incipient fibrosis around the Purkinje fibers and blood vessels. Diffuse congestion was observed in the liver, especially in the centrilobular area. Hepatocytes adjacent to the centrilobular vein had pyknotic nuclei and condensed eosinophilic cytoplasm (necrosis).

IHC was positive for BVDV in lymphoid follicles of the small intestine. The test was negative in the fragments of the other tissues.
DISCUSSION

The diagnosis of BVDV infection in the outbreak was confirmed by detecting the agent in the small intestine (Peyer’s patches). Clinical and pathological findings are consistent with diagnosis of fetal BVDV infection, in this case, resulting in chronic congestive heart failure (CHF) due to myocarditis [25]. No other symptoms described in lethal BVDV infections in cattle [18,33] were observed in the affected calves.

Although cattle CHF associated with BVDV infection has been described [25], detailed reports on the epidemiology, clinical aspects and macroscopic and microscopic lesions caused by this form of infection are not available. Lesions provoked by myocarditis are described in detail for sheep and goat fetuses experimentally infected with BVDV at different gestation stages (41 to 60 days) [7,26], but those lesions were less severe than the ascites, hydrothorax, hydropericardium, hydroperitoneum and nutmeg liver observed in the calf of the present study. Although it was not possible to determine whether the calves were persistently infected (PI), the chronic nature of the cardiac and hepatic lesions indicates that they had been infected during intrauterine life.

A similar phenomenon occurs in poisoning by *Tetrapterys multiglandulosa* in cattle, in which calves born from cows that consumed the plant in early gestation are born apparently healthy but die in the first weeks of life with chronic cardiac and hepatic lesions [13]. In one study, BVDV antigen was detected by IHC in the heart of three aborted or premature goat fetuses with histological lesions and in a fetus without histological lesions [26]. These fetuses aborted or died a few hours after birth, showing no macroscopic changes in the heart; however, histological lesions showed lymphohistiocytic multifocal infiltrates with scattered small areas of myocardial necrosis [7]. These lesions differ from those found in this report, as the necropsied calf was born healthy, died 40 days after birth and presented with more severe macroscopic and histological lesions. In previous reports of myocarditis in cattle associated with BVDV infection, there have been no descriptions of the detection of the antigen by IHC in myocardial lesions. This fact may also explain the lack of detection of the agent in the myocardium, as the injury may have been caused several months before death, allowing time for the subsequent clearing of the agent. Moreover, the absence of correlation between the amount of viral antigen and the location of histological lesions has been described by other authors [29,39].

It is noteworthy that the antigen must be identified in sequential samples collected at intervals of 30 days for an animal to be confirmed PI [8]. It is also noteworthy that the histological findings of heart lesions and the absence of proliferative vascular lesions, which, in cattle, are presumably attributed to persistent BVDV infection, allow the differential diagnosis between BVDV and systemic reactive angioendotheliomatosis-like syndrome [6].

Myocarditis resulting from BVDV infection should be differentiated from injuries to calves born to cows that consumed non-lethal doses of the plant *Tetrapterys multiglandulosa* during pregnancy [13]. These cases are characterized by abortions and the development of weak calves that die soon after birth or following physical exercise. Calves poisoned by
T. multiglandulosa have macroscopical and histological lesions similar to those observed in the present study; however, lesions in poisoned calves are associated with fibrosis, while BVDV-infected calves have lesions characterized by the predominance of infiltrates of lymphocytes and macrophages. Another important histological characteristic differentiating these diseases is the lack of spongy lesions formed by dilation of perivascular spaces or axonal swelling (status spongiosus) in brain white matter in infected animals. This deformation is described in natural poisoning by T. multiglandulosa in cattle and in experimental poisoning in cattle and sheep [12,13].

In areas where Tetrapterys multiglandulosa does not grow, BVDV infections associated with heart lesions should be differentiated from poisoning by other plants, such as Ateleia glazioviana, that cause similar injuries [1,20]. Heart lesions and sudden death can also occur in calves infected with foot and mouth disease virus (FMDV); however, this is a rare form of infection that occurs primarily in outbreaks [28]. Moreover, the herd that was studied was vaccinated, and no viral activity had been reported in the study area.

Differential diagnosis may consider other causes of heart lesions in cattle, such as poisoning by ionophore antibiotics or vitamin E and selenium deficiency [3]. These diseases, however, have not been described in calves several days old, and the symptoms of these diseases include muscular lesions that were not detected in the calves of the present study. Furthermore, the cattle studied had not received ionophores.

Sudden death in cattle following exercise is not rare in Mato Grosso do Sul because the animals commonly consume Mascagnia pubiflora [35]. However, death usually does not occur in 30- to 40-day-old cattle, and macroscopic or histological lesions in the myocardium are not typically seen. Intoxication with this plant causes histological lesions such as vacuolar interface dermatitis of the distal convoluted kidney tubules [42].

Differential diagnoses may also include Neospora caninum infection, which causes nonsuppurative myocarditis, necrosis of certain areas and extensive polymorphonuclear infiltrates in the subendocardial region and around the Purkinje fibers [14]. In addition to heart infection, N. caninum can also damage the nervous system, causing multiple areas of necrosis in both white and gray matter, with or without bleeding and with mononuclear cell infiltrate. N. caninum also causes vacuolization in areas surrounding brain lesions, the presence of eosinophilic spheroids, diffuse gliosis, hypertrophy of the vascular endothelium, the occurrence of perivascular cuffs in mononuclear cells and small areas of mineralization [38]. Neither these lesions nor the presence of intracellular tachyzoites was observed in the case reported.

Viral antigen identification using IHC in Peyer’s patches proved to be a sensitive and specific tool for BVDV detection in cattle. These data confirm previous observations reported in the literature [9,15,21,24]. Given that the antigen was found only in Peyer’s patches, fragments of different organs must be subjected to IHC tests in cases of suspected fetal infection with BVDV. Other studies used the IHC technique to detect BVDV antigen in cattle brain, liver, lung, placenta, spleen, thymus, adrenal glands [4], heart, intestine, kidney, lymphoid tissue, skin [41], esophagus, omasum, thyroid and pancreas [40]. These studies also observed that the antigen is not uniformly distributed among the organs, suggesting that a specific organ of aborted fetuses cannot be chosen for BVDV diagnosis. On the other hand, in a study on the distribution and quantification of IHC markings in different organs of 5 cows persistently infected with BVDV, viral antigens were detected mainly in epidermal keratinocytes, epithelial hair follicles, dermal mononuclear cells of the ears and skin, histiocytes and lymphocytes in the lymph nodes, thyroid follicular cells, neuron cytoplasm and, to a lesser extent, in microglial cells of the cerebral cortex and hippocampus [39].

The marking of hair follicles, dendritic cells and epidermal keratinocytes from ear skin fragments are characteristic of PI animals [9,31,39,40]. Moreover, in cattle with transient infection, the antigen is limited to the epidermis and infundibulum of hair follicles. However, IHC performed on ear skin fragments of two PI calves confirmed by virus isolation was found to be negative [16]. Although more laborious than the sandwich ELISA, the IHC test has the advantage of detecting the BVDV antigen in young cattle without interference from maternal antibodies [9,22]. Thus, skin fragments must also be collected in cases of perinatal and neonatal death.

Although cases of BVDV infection are well documented in Brazil [5,11,17,27,30,32,36], few reports have discussed the economic losses caused
by this infection [16,19], especially in terms of reproductive failure and mortality in neonatal and perinatal periods. A study conducted in Rio Grande do Sul found that only 3 (1.12%) of 161 fetuses examined using the IHC technique were positive for BVDV [40], while another investigation showed that this virus accounts for 2 to 7% of cattle miscarriages in other countries [34].

CONCLUSIONS

This is the first report of nonsuppurative myocarditis associated with BVDV causing perinatal cattle death with agent identification in Mato Grosso do Sul. However, these data are insufficient to determine the importance of BVDV infection in terms of reproductive losses in this state because the methodological approaches used were different from those adopted in earlier studies. For diagnostic purposes, it is essential to systematically collect and analyze samples of all tissues from suspected BVDV cases, especially using IHC tests. Since the antigen is not distributed uniformly, evaluating all tissues increases the likelihood of BVDV detection.

SOURCES AND MANUFACTURERS

1 DAKO kit - LSAB 2 kit, DAKO Corp., Carpinteria, CA, USA.

2 Antibody - primary monoclonal antibody 15C5 (Syracuse Bioanalytical), Syracuse, NY, USA.

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REFERENCES


