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In utero Infection with Bovine Viral Diarrhoea Virus Associated with Neurological Symptoms

Rute Noiva, Maria C. Peleteiro and George Stilwell
Centro de Investigacao Interdisciplinar em Sanidade Animal (CIISA), Nucleo de Patologia e Clinica, Faculdade de Medicina Veterinaria, Universidade Tecnica de Lisboa, Lisbon, Portugal

Abstract: Several new-born pure Limousine calves were presented with neurologic signs that included ataxia and limb hypertonicity/spasticity. These animals showed appetite and were hand fed, soon after birth with colostrum from their dams. BVDV infection was suspected but blood from affected calves was negative to both antigens and BVDV antibodies. No animal in the herd had ever been vaccinated against BVDV. Necropsy and histopathological exam showed some evidence of BVDV infection that was later confirmed by immunohistochemistry. By relying on serology results, most probably affected by colostral antibodies, the correct diagnosis was delayed and adequate measures postponed. This case report shows the importance of knowing the limitations of each diagnostic test in order to correctly interpret results.

Key words: Limousine calves, neurologic sign, limitation, interpret, necropsy, colostrum

INTRODUCTION

The Bovine Viral Diarrhoea Virus (BVDV) is a small, enveloped, positive-sense, RNA virus, belonging to the genus *Pestivirus*, associated with pathology in the respiratory, hematologic, immunologic, neurologic and reproductive systems (Baule *et al*., 2001; Walz *et al*., 2001; Grooms, 2004; Murray *et al*., 2008). Depending on various factors, *in utero* infection with this virus may result in one of five outcomes: early embryonic death, abortion, persistent infection, congenital defects or birth of normal/weak seropositive calves (Grooms, 2004; Radostits *et al*., 2007). Congenital anomalies involving the central nervous system are common following fetal infection with BVDV. These include cerebellar hypoplasia microencephalopathy, hydrocephalus, hydranencephaly, porencephaly and hypomyelination. At birth, calves that have cerebellar hypoplasia show extreme difficulty in becoming ambulatory (Grooms, 2004).

The reproductive losses may be the most economically significant consequence associated with BVDV infection (Grooms, 2006).

CASE DESCRIPTION

This short communication describes a case of BVDV infection in a herd of 55 pure bred Limousine cows, associated with the birth of calves suffering from neurological symptoms. The bull kept at the time with the herd was closely related (same sire) to several heifers and cows. No animal in the herd had ever been vaccinated against BVDV. This case started with the sporadic birth of calves with immediate post partum ataxia and front limb rigidity. The calves were also born with shorter heads and smaller ears than expected for the breed. All animals demonstrated normal appetite and were hand-fed colostrum and milk from their dams but all ended up being euthanized for humanitarian reasons. One calf was sent to a private laboratory (not the researchers) for necropsy and histopathological and microbiological analysis which resulted in a final report of non-suppurative necrosis of the cerebral cortex and isolation of *Salmonella* sp. in the intestine. Microbiological analysis of the water from a nearby stream, aimed at discarding possible environmental causes was negative for major pathogenic organisms. Blood samples were collected from the next three diseased calves, as well as from the mother of one these animals and tested for the presence of anti-BVDV antibodies and BVDV antigens by indirect ELISA and Antigen capture ELISA (ACE), respectively. All calves were negative on both indirect ELISA and ACE. The cow was positive on ELISA but negative on ACE. Several months after the birth of the first affected calf due to the persistency of the problem and lack of a definitive diagnosis, two calves were sent to the Faculty of Veterinary Medicine in Lisbon. The first calf was 8 days old and presented ataxia, hypertonicity/spasticity of the front and hind limbs (without loss of deep sensitivity),

Corresponding Author: George Stilwell, Centro de Investigacao Interdisciplinar em Sanidade Animal (CIISA), Nucleo de Patologia e Clinica, Faculdade de Medicina Veterinaria, Universidade Tecnica de Lisboa, Lisbon, Portugal

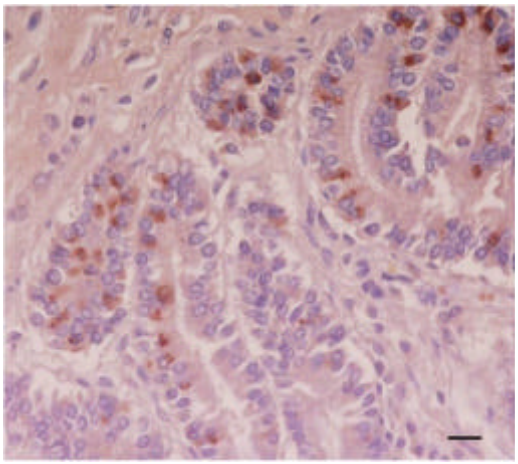

Fig. 1: Immunohistochemical staining for BVDV antigens in small intestine section (ABP, Mayer's Hematoxylin). Bar = 100 μm

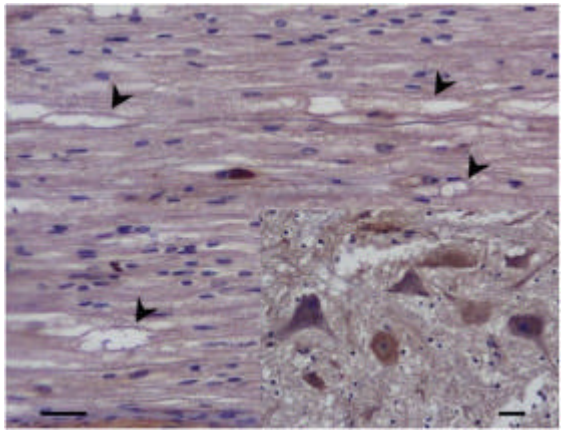

Fig. 2: Immunohistochemical staining for BVDV antigens in brachial plexus nerve and spinal cord sections. Wallerian degeneration is also evident (arrowheads) (ABP, Mayer's Hematoxylin) Bar = 100 μm

opisthotonus and hyperestesia of the back and loin. Palpebral and corneal reflexes were present with no loss of vision. As with the previous cases, the head and ears were shorter than expected. The animal was euthanized, showing, at necropsy, signs of mild congestive enteritis. Examination of the brachial plexus revealed oedema of the perineurium. Several microglial cell infiltrates were present in the thalamus and cerebral cortex, along with some sub-epithelial infiltrates in the lateral ventricles. The cerebellum and brainstem revealed hypomyelination. Cerebrospinal fluid was collected at necropsy and sent for microbiological analysis. Bacteria belonging to the genus *Oerskovia*, *Staphylococcus* and *Acinetobacter* were isolated. However, due to the early onset of the disease these were considered sample contaminants.

The second animal was 2 days old and had been killed at the farm. At necropsy and histopathological analysis, signs of severe enteritis with massive epithelial shedding and bacterial aggregates in the lumen were observed. The brachial plexus exhibited Wallerian degeneration and the adipose tissue surrounding them showed diffuse haemorrhaging. Neuritis and perineuritis, along with necrosis of the nerve sheath cells were observed in the rachidian nerves. In the encephalon, focal subependimary lymphoid infiltration of the lateral ventricles and rare inflammatory infiltrates, occasionally with perivascular disposition were seen.

Although, inconclusive, the results of both necropsies suggested the possibility of *in utero* infection with BVDV. A few months later, 4 μm sections of intestine and nervous tissue from both calves were analysed by immunohistochemistry. The sections were incubated with anti-BVDV goat policlonal antibody (VMRD, Inc.) for 60 min. A commercially available immunoperoxidase labelling system used (Vectastain® GoatIgG ABC Elite kit; Vector Laboratories), modified to better control cross-reactivity was used. Positive staining, in the form of a granular perinuclear cytoplasmic brown precipitate in the intestinal epithelial cells was observed for both animals (Fig. 1). The same precipitate was seen in the cytoplasm of peripheral nerve sheath cells, in the brachial plexus and of neurons in the spinal cord (Fig. 2). This confirmed the presumptive diagnosis of in utero infection with BVDV, although, it was not possible to classify these animals as persistently infected.

DISCUSSION

Based on the clinical signs the first presumptive diagnosis for these cases was *in uterus* BVDV infection. Although, cerebellar hypoplasia was not present, hypomyelination found in several CNS structures was thought to be responsible for the ataxia and other neurologic signs. However, the ELISA results apparently contradicted the BVDV infection diagnosis so other causes were sought. The spasms, hyperesthesia and ataxia, as well as the neuraxial oedema found in one of the calves was very similar to a congenital condition described in Hereford new-borns (Rousseaux *et al*., 1985; Duffell, 1986; Harper *et al*., 1986). The same condition has been described in Holstein-Frisian (Bethlehem *et al*., 1992; Schulze *et al*., 2006) because the degree of inbreeding that is described in the published cases was similar to what was happening in the herd a genetic cause for the problem

was proposed. As a result the bull was culled. Until the IHC was performed in several tissues collected from the two animals brought to the faculty and BVDV infection was confirmed, no control program or vaccination was implemented in the herd. This may have allowed for further reproductive losses.

The apparent incompatibility between the results obtained by IHC, ELISA and ACE could be explained by the presence of colostral antibodies. Zimmer demonstrated that the antigen ELISA test was shown to be unreliable indicator for the diagnosis of infections with BVDV when used in the presence of high levels of maternal antibodies. These may react with circulating virions, forming immune complexes and making both the antibodies and the viral antigen undetectable by ELISA and ACE (Brock *et al*., 1998; Saliki and Dubovi, 2004; Cornish *et al*., 2005). In view of the fact that the detection of viral antigen in serum or peripheral blood leucocytes in the presence of circulating antibodies is at least unpredictable, IHC seems a consistent alternative. This method has other advantages for the early detection of heavily BVDV infected animals including ease of sample collection and transport to the laboratory.

CONCLUSION

Microopthalmia (Kahrs *et al*., 1970) and mandibular brachygnathism (Scott *et al*., 1972) have been reported but to the knowledge, this is the first published description of calves born with small head and ears due to fetal BVDV infection. This case report stresses the importance of knowing the bases and limitations of each diagnostic test in order to correctly interpret results and reach a reliable and prompt diagnosis.

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