Shocking shaking

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Introduction

Bovine viral diarrhea (BVD) is one of the most prevalent infections in cattle. The virus, a Pestivirus of the family *Flaviridea*, can cause a serious clinical disease. Its complex pathogenesis and different timing of infection, during pre- or post- gestational period, affect the severity at presentation. A dam's infection with BVD during pregnancy may have different outcomes for the fetus, including abortion, premature/stillbirth, congenital malformations, or subclinical forms. Infections during the first gestational trimester may result in viral persistence and immunotolerance to BVD virus (BVDV) in the fetus leading to Persistently Infected (PI) calves. Moreover, although PI animals could appear clinically healthy and survive in the herd, some of them may actually be small, weak and ill-thrifty, showing higher predisposition to mucosal disease and secondary infections due to poor immune function (1). Finally, the fetus becomes immunocompetent between 100 and 150 days of gestation; during this time, BVD infections could result in teratogenic effects, typically affecting eyes, bones, haircoat and the brain, frequently leading to cerebellar dysplasia (2).

The aim of this report was to describe an outbreak of BVDV infection during pregnancy resulting in PI calves with tremor due to hypomyelination of the central nervous system (CNS). This complication has rarely been reported in literature.

Farm Facts

Between May and September 2016, 12 out of 45 calves (Table 1) presented with generalized tremors at birth in a northern Italian Friesian farm. This represents a typical commercial dairy farm, of 140 milking cows, with an average milk production of 33 kg/day. The farm produces forage and buys proteic TMR from a local feed mill industry. Farmers never acquire animals from other cattle breeding facilities. Pregnancy is obtained only from artificial insemination of heifers and cows with different bulls' semen, showing good reproductive performance with an abortion rate of around 3% and calf mortality of about 4%.

Vaccination against the most common infectious diseases spread in the area, such as Infective Bovine Rhinotracheitis (IBR) and BVD, had not been used at the time.

Case

In May 2016, a few calves presented heterogeneous neurological manifestations at birth. The symptoms included generalized tremors, muscle fasciculations, ataxia, and different grades of disability at assuming and maintaining quadrupedal stance. Deambulation was characterized by generalized ataxia and hypermetria. Horizontal or vertical nystagmus was observed. Organic functions and suction of colostrum and milk were preserved. Spinal and cranial nerve reflexes, papillary re-

CASE	BIRTH	Calf sex	State	Insemination date	COW Ab NS2-3[1]	CALF Ab NS2-3[1]	CALF ELISA Ag[2]	CALF ELISA Ag[3]	EAR NOTCH[4]	CALF RT-PCR[5]
1	10/07/2016	F	ILL	08/10/2015	+	/	/	/	/	/
2	18/07/2016	M	ILL	20/10/2015	+	+	+	+	/	/
3	18/07/2016	M	ILL	20/10/2015	+	+	+	+	/	/
4	04/08/2016	M	ILL	01/11/2015	+	+	+	+	/	
5	08/08/2016	F	ILL	09/11/2015	+	+	+	+	/	/
6	09/08/2016	M	ILL	15/11/2015	+	/	+	/	/	/
7	11/08/2016	F	HEALTHY	13/11/2015	/	/	/	/	+	+
8	27/08/2016	F	ILL	22/11/2015	/	+	+	/	/	/
9	28/08/2016	F	ILL	23/11/2015	/	-	/	/	+	/
10	03/09/2016	F	ILL	06/12/2015	/	/	/	/	+	/
11	08/09/2016	F	ILL	08/12/2015	/	/	/	/	+	+
12	09/09/2016	M	ILL	09/12/2015	/	/	/	/	+	/
13	24/09/2016	F	ILL	16/12/2015	/	/	/	/	+	/
14	03/04/2017	F	ILL	03/07/2016	+	-	+	+	+	+

Table 1. Characteristics and diagnostic tests performed on calves and their dams. ([1]) ELISA for Antibodies anti non structural proteins 2-3 in serum. ([2]) ELISA for BVDV antigen in serum. ([3]) ELISA for BVDV antigen in viscera. ([4]) ELISA antigen for BVDV in auricular cartilage. ([5]) RT- PCR for BVDV on serum. (+): positive test. (-): negative test. (/): test not performed.

flex, muscle strength, menace and nociceptive reflexes were not compromised.

Symptomatic calves did not receive any pharmacological treatment and, with time, became unable to support themselves. Considering the poor prognosis, the owners later decided to euthanize newborn neurological symptomatic calves in the first days of life.

Thereafter speculations about etiology of the described cases were discussed, leaning toward the hypothesis of an infectious pathogenesis. Cow and calves were therefore tested for Bovine Herpes Virus type 1 and 3 (BHV-1 and 3), BVDV, Schmallenberg virus, and Neospora Caninum. Calves number 2, 3, 4 and 5 were thoroughly examined postmortem at Bologna Veterinary University, while case number 14 was examined at Cremona IZS laboratories.

Tests were carried out on samples from blood, viscera, and auricular cartilage (ear notch) of the affected calves. Serological analyses in dams showed positivity for NS2-3 antibodies against BVDV. Real Time Protein Chain Reaction (RT-PCR) and Enzyme-linked immunosorbent assay antigens (ELISA Ag) detected BVDV in blood and viscera. ELISA was performed on all newborn calves' ear-notch samples to uncover possible asymptomatic PIs, revealing the presence of antigen in case

number 7. Bologna University laboratory detected BVDV RNA type 1 subtype b from a subclinical PI and then compared the result with RNA virus extracted from symptomatic PI calves, showing a match of 99.7-100% nucleotide identity.

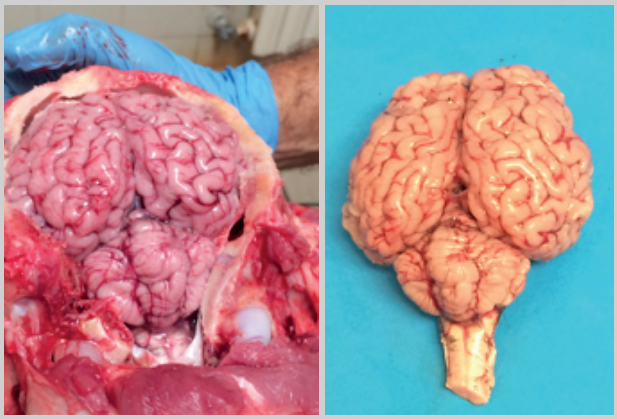
Furthermore, all viscera of the five symptomatic calves that were examined postmortem, including brain and spinal cord, presented mascroscopically normal at necroscopy (Figures 1 and 2). Representative portions of brain and spinal cord were processed for histological analysis and stained with myelin specific Luxol fast blue detecting diffuse neuroaxial hypomyelination (Figures 3 and 4). Calves presented microscopic findings characterized by severe and diffuse deficiency of myelin and different degrees of multifocal gliosis and malacia associated with signs of inflammation, such as swollen axons and neoangiogenesis of cerebellar peduncle and medulla oblongata.

Of note, calf number 14 resulted positive at birth for BVDV type 1. For diagnostic confirmation the calf was euthanized at three weeks of life and virological analysis were performed on brain and viscera; BVDV type 1 was isolated on cellular culture Madin-Darby Bovine Kidney (MDBK) from brain, lungs, spleen and kidneys. Comparable results were obtained when using Rabbit Embryonic Brain (REB) cellular culture. The presence of antigens and virus in the brain indicated congenital persistent infection (2).

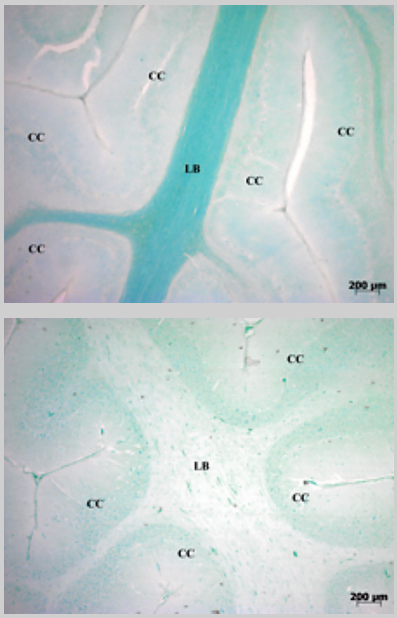
In order to prevent new infections in pregnant cows, from October 2016 all animals received immunization against BVDV with a modified live virus vaccine, starting at 3 months of life. For one year, ELISA was performed on all newborn calves' ear notch. Bulk tank milk sample was tested for BVDV with RT-PCR every four months. Only case number 14 was positive, likely due to the infection of the fetus during the first trimester of gestation before the dam received vaccination.

Conclusion

This case series presents an outbreak of BVD in an Italian dairy herd which resulted in a high incidence of PI calves showing neurological symptoms at birth due to natural acquired intrauterine infection with BVDV.



Figures 1 and 2. Post mortem exam of calf with neurological manifestations showed no macroscopic lesions.



Figures 3 and 4. Calf cerebellum, histological section of Cerebellar Lamellae (CC) Lamina Basalis (LB) in healthy (top) and infected (bottom) calves, stained with Luxol Fast Blue. PI calf infected by BVDV presented severe and diffuse myelin deficiency.

Both Border Disease Virus and Bovine Viral Diarrhoea Virus belong to the Pestivirus family. Although tremors and hypomyelination are a common sequela of in utero Border disease virus infection in sheep (2), such neurological manifestations of BVDV infection have rarely been described. Otter et al reported BVDV-associated disease in 23 herds in England and Wales between 1991 and 2007 with congenital tremors and hypomyelination as predominant lesions.

Atypical presentation of BVD-related disease may be explained by difference in timing of infection during pregnancy, strain of virus, infective dose, breed, age, and immune status of the dams (2). Foetal development stage may be held responsible for pathological effects of the infection. The predominant lesion observed was hypomyelination, while calves did not present the most classic teratogenic effects of BVDV, such as cerebellar hypoplasia and dysgenesis (3). Hypomyelination in calves is likely a consequence of infection at the end of the first trimester of gestation, when the fetus is not able to mount an inflammatory response because of incomplete maturation of immune competence (2, 4, 5). It remains unclear how the virus was first contracted into the herd and why one PI calf did not present with neurological signs and pathogenesis of hypomyelination.

Timewise, the contagion must have first occurred between January and February 2016 when the farm registered an increased rate of abortions, embryonic deaths, and post-partum complications. BVDV can survive outside the host for not more than a few weeks in optimal conditions with favorable pH and temperature. Considering farm location in a high density area of cattle breeding, vectors of infection might be visitors, livestock transport or droplets.

Although classical BVD usually presents subclinically at birth, we observed only one case of PI calf without neurological manifestations potentially representing the heterogeneity of manifestations of the same virus strain (2).

Nevertheless, hypomyelination could be the result of interference in oligodendrocyte differentiation, as has been suggested for Border disease in lambs (6).

Further research will be necessary to understand the mechanism of infection of this particular BVDV strain. Genotype analysis will be performed to explain why the same viral strain did not cause neurological manifestations in one case only.

References

1. Sasha R. Lanyon, Fraser I. Hill, Michael P. Reichel, Joe-Brownlie (2014). Bovine viral diarrhoea: Pathogenesis and diagnosis. *The Veterinary Journal*. 199: 201-209.
2. Otter A, de B Welchman D, Sandvik T, Cranwell MP, Holliman A, Millar MF, Scholes SFE (2009): Congenital tremor and hypomyelination associated with bovine viral diarrhea virus in 23 British cattle herds. *Vet Record* 164: 771-778.
3. Gallina, Ciulli, Bombarsi, Mandrioli, Ghilardi, Scagliarini, Gentile (2017). Congenital tremor and hypomyelination associated with transplacental infection with bovine viral diarrhea virus (BVDV) in an Italian cattle herd. *Abstract Sisvet* 2017.
4. Duffell, S. J. & Harkness, J. W. (1985). Bovine virus diarrhoea-mucosal disease infection in cattle. *Veterinary Record* 117, 240-245.
5. Nettleton & Entrican (1995). Ruminant pestiviruses. *British Veterinary Journal* 151, 615-642.
6. Binkhorst G. J., Journée D. L. H., Wouda W. , Strayer P. J. & Vos J. H. (1983). Neurological disorders, virus persistence and hypomyelination in calves due to intra-uterine infections with bovine virus diarrhoea virus, *Veterinary Quarterly*, 5:4, 145-155.