The first finding of the ophthalmosteresis in a Korean native calf with bovine viral diarrhea - case report

Giyong Seong¹, Jae-Ku Oem², and Kyoung-Seong Choi¹*

¹Department of Animal Biotechnology, College of Animal Science, Kyungpook National University, Sangju, South Korea
²Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Anyang, South Korea

ABSTRACT
A case of ophthalmosteresis associated with bovine viral diarrhea virus (BVDV) infection in a female Korean native calf is identified. This congenital anomaly is rare in cattle. Clinical examination revealed no further anomalies. The presence of BVDV antibodies was observed in serum. This is the first documented case of ophthalmosteresis in a calf specifically attributed to BVDV infection.

Key words: ophthalmosteresis, bovine viral diarrhea virus, antibodies

Introduction
Bovine viral diarrhea virus (BVDV) has an important impact on the productivity of the cattle population and is a major cause of economic loss in the cattle industry worldwide. BVDV infection in susceptible pregnant cows leads to transplacental spread of the virus. The outcomes of fetal infection vary from abortion, birth of a weak or undersized calf, persistent infection, or to congenital defects, depending on the biotype and virulence of the infecting virus, the immune status of the host, and the timing of infection (BROWNLIE, 1991; FRAY et al., 2000; MOENNIG and LIESS, 1995)

Congenital defects associated with BVDV infections may involve microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, and hypomyelination, lenticular cataracts, retinal degeneration, optic neuritis, microphthalmos, chorioretinopathy, alopecia, hypotrichosis, brachygnathia, growth retardation, arthrogryposis, or thymic aplasia.

*Corresponding author:
Kyoung-Seong Choi, DVM, MS, PhD, Department of Animal Biotechnology, College of Animal Sciences, Kyungpook National University, 386 Gajang-Dong, Sangju 742-711, South Korea, Phone: +82 54 530 1222; Fax: +82 54 530 1229; E-mail: ksoh03@knu.ac.kr
Here, we describe the occurrence of a congenital anomaly in a Korean native calf, with serological evidence of transplacental BVDV infection.

**Case presentation**

In June 2013, a newborn female Korean native calf was born without any eyes. The calf presented a congenital malformation in which the eye sockets were very ill-developed and the orbit rudimentary was absent, and it revealed apparent blindness as a result of ophthalmic examination. The veterinarian made the diagnosis of ophthalmosteresis, namely the absence of both eyes (Fig. 1). No other clinical signs were observed. The animal had a normal appetite and was tube-fed milk, because it was unable to move unassisted from birth and adopted a continual crouched posture in the barn. Blood samples were collected before the initial feeding. Three other calves born to the same mother cow were normal. The herd had not been routinely vaccinated against BVDV, but this unusual malformation was a one-off occurrence; there were no other indications of BVDV infection in the herd. Hematological analyses were performed on the mother (4 years old) and calf (1 day of age) (Table 1). In the mother, severe thrombocytopenia and leukocytopenia were observed. No hematological abnormalities apart from anemia were detected in the calf. All virological tests for Akabane and Chuzan were negative. EDTA-treated blood samples were tested for BVDV antigens by ELISA (IDEXX Laboratories Inc., Westbrook, ME, USA), but antigen-capture ELISA showed a negative result. Antibodies to BVDV in serum were detected by ELISA, using a Svanovir® BVDV-Ab ELISA kit (Svanova Biotech AB, Uppsala, Sweden) in the mother and this calf. Serum neutralization antibody titer to the NADL strain was performed using a microtitration-based assay (MAISONNAVE and ROSSI, 1982). Briefly, after the serum was heat-inactivated at 56 °C for 30 min, two-fold dilutions (1:2-1:1024) of serum (50 µL) were incubated with equal volumes of NADL strain, and cytopathic BVDV-1 (100 TCID₅₀). After incubation for 1 h at 37 °C, 50 µL of Madin-Darby bovine kidney (MDBK) cells were then added to each well of a 96-well plate. The presence or absence of a virus-induced cytopathic effect was observed after 4 days of incubation. RT-PCR was performed on the blood, but this animal was BVDV negative (CHOI and SONG, 2011). The results for BVDV detection are summarized in Table 2.
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Fig. 1. Photograph showing the absence of left (A) and right (B) eyes

Table 1. Summary of clinical pathologic findings and BVDV detection results for calf showing congenital anomaly and mother cow

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calf (1 day)</th>
<th>Mother cow (3 yrs)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (K/mL)</td>
<td>6.52</td>
<td>4.67</td>
<td>6-17</td>
</tr>
<tr>
<td>WBC differential</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (K/mL)</td>
<td>2.28</td>
<td>2.9</td>
<td>3-12</td>
</tr>
<tr>
<td>Eosinophils (K/mL)</td>
<td>0</td>
<td>0.007</td>
<td>0-1.9</td>
</tr>
<tr>
<td>Lymphocytes (K/mL)</td>
<td>3.04</td>
<td>1.55</td>
<td>0.5-4.8</td>
</tr>
<tr>
<td>Monocytes (K/mL)</td>
<td>1.08</td>
<td>0.19</td>
<td>0.1-2.8</td>
</tr>
<tr>
<td>RBC (M/mL)</td>
<td>8</td>
<td>6.24</td>
<td>5.5-8.5</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>10.1</td>
<td>12.6</td>
<td>12-18</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>27.8</td>
<td>34</td>
<td>37-54</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>34.7</td>
<td>54.5</td>
<td>64-74</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>12.6</td>
<td>20.1</td>
<td>22-27</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>36.3</td>
<td>36.9</td>
<td>34-36</td>
</tr>
<tr>
<td>PLT (K/mL)</td>
<td>1559</td>
<td>64</td>
<td>160-430</td>
</tr>
<tr>
<td>BVDV antigen ELISA (blood)</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>BVDV antibody ELISA</td>
<td>Positive</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of BVDV detection in calf with congenital malformation

<table>
<thead>
<tr>
<th></th>
<th>RT-PCR</th>
<th>Ag-ELISA</th>
<th>Ab-ELISA</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>1.624*</td>
<td>32</td>
</tr>
</tbody>
</table>

*Ab-ELISA optical density values, 0.20 were considered positive
Discussion

Most fetal BVDV infections result in persistent subclinical infection, fetal death, or congenital defects. The ophthalmosteresis described in this case is considered as a rare manifestation of in utero BVDV infection in cattle. Possible reasons for these manifestations include the stage of gestation at the time of infection, differences in virus strain, and other factors, such as breed, age and immune status of the dams.

Ophthalmosteresis and the presence of BVDV antibodies examined in this case were explained by congenital developmental defects caused by transplacental BVDV infection, which can cause congenital defects in newborn calves (BLANCHARD et al., 2010; PORTER et al., 2010). Congenital defects, such as retinopathy, cerebella dysgenesis and haircoat abnormalities, may occur in animals infected between 100-150 days of gestation, when the fetus is capable of mounting an inflammatory response and the development of the central nervous system (CNS) and the eyes are in its final stages. At this time, BVDV infects a wide range of fetal tissues, and thus interferes with growth, differentiation, tissue maturation, and the evolving immune system (MOENNIG and LIESS, 1995; OTTER et al., 2009). The diagnosis of congenital developmental defects by transplacental BVDV infection is based on the presence of characteristic clinical signs and on the detection of the BVDV antigen or RNA. Moreover, BVDV antibody detection in serum collected from a calf before it has ingested colostrum is an important diagnostic tool of transplacental infection.

In the described case, BVDV antibodies were found in serum collected before ingestion of colostrum from the calf, and serum from its mother. However, the viral antigen was not demonstrated by both ELISA and RT-PCR, which suggests the viral antigen had been cleared by BVDV antibodies. Furthermore, this calf developed a substantial neutralizing antibody titer in its serum due to high levels of maternally derived antibodies, as determined by a virus neutralization test (Table 2). This can be explained by the evidence that a prenatal fetal BVDV infection occurred. The outcome of BVDV in utero infection depends on the timing of the infection. Infections before the calf has become immune competent at 150 days of gestation can result in abortion, congenital defects, or persistent infection, whereas those occurring after 150 days are typically cleared (DONE et al., 1980). The outbreak of ophthalmosteresis shown in this case was associated with BVDV infection, and is a rare congenital anomaly.

Conclusion

In the present study, ophthalmosteresis associated with congenital BVDV infection is an uncommon outcome. The presence of BVDV antibodies in the serum of the anomalous calf can be considered as the result of in utero infection. BVDV infects cows at a critical stage of gestation, and the calf could be susceptible to infection at the time of exposure.
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The time of infection of the fetus in this case cannot be determined exactly, but the ophthalmosteresis observed in this calf was probably caused by natural in utero BVDV infection, which can result in this congenital anomaly before 150 days of gestation. This is the first description of a calf born with ophthalmosteresis due to transplacental BVDV infection.

Acknowledgements
This work was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF 2012R1A1A3011238).

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Received: 3 January 2014
Accepted: 25 November 2014


SAŽETAK


Ključne riječi: ophthalmostereza, virus virusnog proljeva, protutijela, tele