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Outbreak of Bovine Viral Diarrhea Virus in Korean Indigenous Calf

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Abstract : A 25-day-old, Korean indigenous calf was presented with a 10 days history of respiratory disorders and bloody diarrhea, and died. This calf was extremely unthrifty compared to others and had evidence of chronic diarrhea based on matting of feces in the hair of the tail and perineum. Ecchymotic hemorrhages were observed on multiple organs at necropsy. Bovine viral diarrhea virus (BVDV) infection was identified by RT-PCR. The phylogenetic analysis showed that this case belonged to BVDV-2a subgroup and was related to highly pathogenic USA isolate 890 (U18059). This case provided evidence for circulation of BVDV-2 in Republic of Korea. The occurrence of BVDV-2 was also reconfirmed.

Key words **:** Korean indigenous calf, bovine viral diarrhea virus, phylogenetic analysis, BVDV-2a.

Introduction

Bovine viral diarrhea virus (BVDV) is an economically important worldwide disease and one of the most significant viral pathogens in the livestock industry that causes fatal diarrhea syndrome, respiratory disorders, and reproductive failure. The clinical signs of BVDV-infected cattle range from inapparent to severe hemorrhagic syndrome with a high mortality rate (10,12). Infections of pregnant cows is often associated with reproductive losses, including embryonic or fetal deaths, abortions, congenital malformations, stillbirths, and the birth of immunotolerant calves that are persistently infected (PI) (3,7). PI animals are reservoirs of infection that shed virus in most all secretions throughout their lifetime and infect the herds (4,15,17). Thus, PI calves have a significant impact on herd productivity.

The 5'-untranslated region (UTR) is highly conserved and has been used to define genotypes. The phylogenetic analysis based on the 5'-UTR showed that BVDV could be divided into BVDV-1 and BVDV-2 (11,18,19). While BVDV-1 is widely spread in the world and associated with mild clinical signs, BVDV-2 was reported in the United States, Canada, Europe, South America, and Japan, and has been highly pathogenic characterized by thrombocytopenia and severe hemorrhagic syndrome with high mortality (1,8,10,14).

Recently, severe acute BVDV outbreaks among veal calves were observed and the mortality rate was reported with increasing frequency, which cause severe economic losses to farmers in Republic of Korea. In this case report, we describe the genetic characterization of BVDV case from dead calf with a history of diarrhea and respiratory problems.

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Case

A Korean indigenous calf (25 days, female) was presented with a 10 days history of progressively worsening signs of respiratory disorders (fever, inappetence, coughing, and nasal discharge) and bloody diarrhea that were aggravated with gaunt and dehydration, and followed by death in June, 2009. This calf was extremely unthrifty compared to others and had perineal soiling suggestive of chronic diarrhea. Information on the BVDV vaccination was not available, because the pregnant cow was purchased in May, 2009 and calved after 1 month. At necropsy, ecchymosis was observed on multiple organs. The lungs were hemorrhagic and scattered spots of black pigments. Tissue samples (liver, lung, small intestine, and spleen), rectal swab, and nasal discharge were collected and immediately frozen at -80°C until used. Bacteria were cultured and significant bacteriologic isolates were not found. A suspect case was submitted for BVDV diagnosis and tested for the identification of BVDV by RT-PCR. This farm originates from Sangju city, Gyeongbuk province, which has a cattle population of about 600 heads.

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) from tissues, and nasal/rectal swabs. For RNA extraction, nasal/rectal swab samplings were made into 50% suspensions (v/v) using PBS. RT-PCR was performed with Superscript™ One-Step RT-PCR System with Platinum *Taq* (Invitrogen, USA) according to the instructions of the manufacturer. Amplification and sequencing of 5'-UTR was performed using 324 and 326 primers as previously described (16). The predicted size of the amplified PCR product was 288 bp. For amplication, reverse transcription was performed at 50°C for 30 min, and pre-PCR denaturation was performed at 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. RT-PCR

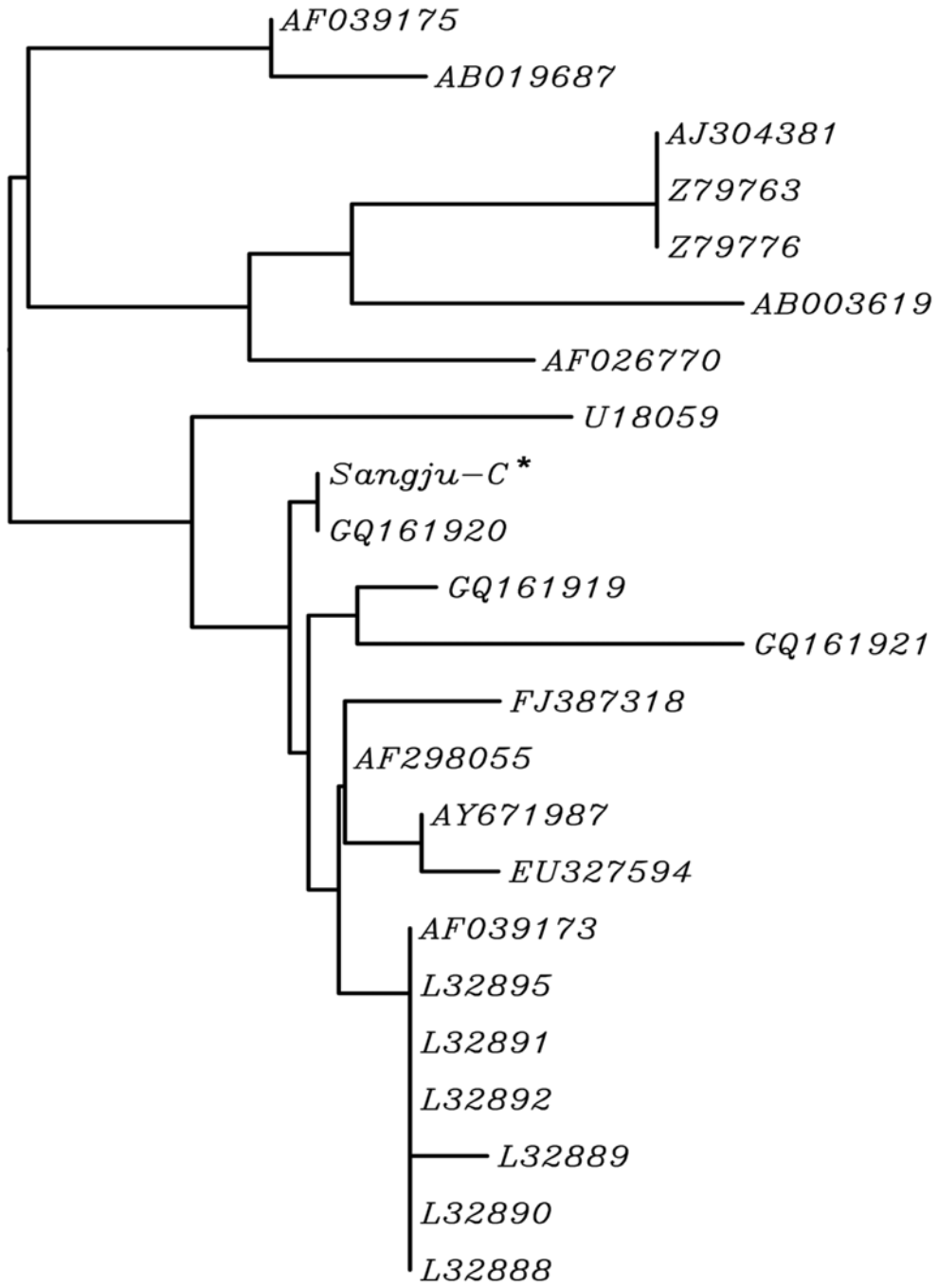

Fig 1. Phylogenetic analysis of BVDV-2a isolates based on the 5'-UTR sequences. An unrooted neighbor-joining tree was constructed from 23 genome sequences. Bootstrap values are indicated as a percentage for 100 replicates A case sequenced in this study is indicated in an asterisk. GenBank accession numbers GQ161919-GQ161921 were which we previously submitted.

products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide.

The PCR products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA) The nucleotide sequences were determined by direct sequencing of the PCR products using BigDye terminator cycle sequencing kit (Applied Biosystems, Foster, USA) and analyzed on ABI PRISM® DNA analyzer (Applied Biosystems). The sequences data were aligned initially using the Clustal X (1.60) (13). Additional sequences from representative isolates of previously identified BVDV-2 were obtained from GenBank and included with each set of alignments. Phylogenetic analysis was constructed using the neighbor-joining (NJ) method with 100 replications in the bootstrap analysis. Displaying trees were drawn with the Treeview program (9).

Discussion

BVDV infection was detected in all samples by RT-PCR. While most BVDV infections occurred in late winter and in spring, this case occurred in June. The maternal blood was collected and examined for BVDV infection. Three weeks later, the mother cow was tested again and identified as PI. Both nucleotide sequence homology was 100% identical. Sequencing analysis showed that our case was 99.7% nucleotide sequence identity with GQ161920 which we previously reported. The phylogenetic analysis revealed that this BVDV field case belonged to BVDV-2a subgroup, and this case was related to USA isolate (U18059, 890) and highly pathogenic (Fig 1). This result indicates that BVDV-2 infections represent a higher early fetal infection than BVDV-1, and BVDV infections in calves cause to a severe economic loss to farmers.

Although the prevalence and economic impact of BVDV infection have been highlighted, the eradication of BVDV is very difficult. PI animals have an important role in the perpetuation of BVDV infections. These animals multiply BVDV at a high rate of months or years and have been described as BVDV producing factories (5). In this farm, the number of ill-thrift and deaths in calves increased within the past 6 months. However, the owner did not agree to examine individual cows for further diagnostic investigations. The main source of BVDV infection for this farm might be cows purchased. Most of these cows were pregnant and calved on the property soon after purchase. Some purchased cows might be persistently infected and the calves were the most likely source of BVDV infection. It is possible that the pregnant cows were first exposed to BVDV in the sale yard and the contamination of pregnant females by a permanent excretor may result in the generation of new PI. PI calves have low survivorship and may no longer be alive. Therefore, it is important to diagnose the significance of cow as natural reservoir of BVDV PI and prevent associated risk of infection to calf.

BVDV infections in calves cause immunosuppression. This may result in the outbreak of infectious diseases and increase in occurrence of secondary or opportunistic infections (2,6). It has been reported that clinical manifestations of diarrhea or pneumonia in calves progressed to severe or chronic state. Consequently, the total economic loss of this outbreak was considerable. Thus, early detection and eradication might prevent BVDV infection on a farm. Further research should focus on the genetic characterization of BVDV sequences from different regions of Republic of Korea.

In conclusion, this case report indicated that BVDV-2a is the predominant subgroup and widespread in Korean indigenous cattle. BVDV infection should not be overlooked as a possible cause of economic losses in livestock industry. This result may contribute for a better understanding of the epidemiology and pathogenensis of BVDV infection.

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한우송아지에서 소 바이러스성 설사병 바이러스 발생

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요 약 : 25일령의 한우송아지가 10일 간의 호흡기질환과 출혈성 설사의 병역을 나타내다가 폐사하였다. 이 송아지는 다른 송아지들과 비교해보았을 때 극도로 위축되어 있었다. 꼬리와 회음부의 분변 흔적은 만성설사가 지속되었음을 말해주었다. 부검시 다양한 기관(조직)에서 반상출혈이 관찰되었다. 역전사 중합효소연쇄반응에 의하여 소 바이러스성 설사병 바이러스가 진단되었다. 이 증례는 계통발생분석에서 BVDV-2a 그룹에 속하는데, 이것은 고병원성인 미국 균주 890 (U18059)과 유사하다. 본 증례는 BVDV-2가 한국에서 유행하고 있다는 증거를 제공한다. 이를 통해 우리는 BVDV-2의 발생을 재확인하였다.

주요어 : 한우송아지, 소 바이러스성 설사병 바이러스, 계통발생분석, BVDV-2a.