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Phylogenetic Analysis of Bovine Viral Diarrhea Virus from Nasal Swab Sample of Persistently Infected Cattle in Republic of Korea

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Abstract : Bovine viral diarrhea virus (BVDV) is an economically important worldwide disease in livestock industry. In this study, the occurrence of BVDV in Korean indigenous cattle was performed by RT-PCR using nasal swab. Twelve of 21 cattle were identified as BVDV positive and classified as persistently infected (PI). These animals showed the occurrence of diseases such as diarrhea and pneumonia. BVDV PI outbreaks were found mostly in PI calves. Sequencing and phylogenetic analysis based on the 5'-untranslated region (UTR) showed that our case belonged to BVDV-2a. These results suggested that the nasal swab sampling was available method for the detection of PI animals, underscoring the need for BVDV control strategies in Korean indigenous cattle.

Key words **:** Bovine viral diarrhea virus; Nasal swab; Persistently infected; BVDV-2a.

Introduction

Bovine viral diarrhea virus (BVDV) is the etiological agent of bovine viral diarrhea-mucosal disease (BVD-MD) and has been distributed worldwide. The clinical signs of BVDV-infected cattle range from inapparent to severe hemorrhagic syndrome with a high mortality rate (8-10). The 5'-untranslated region (UTR) is highly conserved and has been used to differentiate genotypes. The phylogenetic analysis based on the 5'-UTR showed that BVDV could be divided into BVDV-1 and BVDV-2 (8,11,15).

Cattle infected by BVDV in early gestation can give birth to immunotolerant calves that are persistently infected (PI). These PI animals are major reservoirs of infection, shed virus in most all secretions throughout their lifetime, and infecting herds (2-5). PI cattle show varied clinical manifestations such as diarrhea, pneumonia, poor growth, some succumb to mucosal diseases, and some PI cattle do not show clinical manifestations. These PI animals are suspected as the increase in occurrence of secondary or opportunistic infections (1,6), causing serious economic losses to the livestock industry. Therefore, it is very important to identify and remove PI animals from the cattle herd. The objective of this study was performed to investigate the identification of PI cattle using nasal swab sampling and to characterize these Korean BVDV PI cases by nucleotide sequencing and phylogenetic analysis.

Cases

The nasal discharges were collected between January and April in 2009 from 21 Korean indigenous cattle with diarrhea and respiratory problems on 3 different farms that originated from Youngju city, Gyeongbuk province, Republic of Korea (ROK). These farms had no history of BVDV PI animal detection and did not show the outbreak of diseases in the past 6 months. BVDV was detected by the nasal swab sampling. To assess whether BVDV infection was transient or persistent, blood samples were collected from all positive animals and examined again after three weeks using RT-PCR.

Nasal swabs were suspended in 500 μl of PBS and used for RNA extraction. Total RNA was extracted using Trizol Reagent (Invitrogen, USA) according to the instructions of the manufacturer. RT-PCR was performed with Superscript™ One-Step RT-PCR System with Platinum *Taq* (Invitrogen, USA) according to the manufacturer's protocol. Amplification and sequencing of 5'-untranslated region (UTR) was performed using 324 and 326 primers as previously described (14). The forward (324) and reverse (326) primers used were 5'-ATG CCC WTA GTA GGA CTA GCA-3' (W = A or T) and 5'-TCA ACT CCA TGT GCC ATG TAC-3'. For amplification, 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 s, 55°C for 30 s, and 72°C for 1 min. RT-PCR products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide. All samples were tested individually.

The PCR products were purified for direct sequencing using QIAquick PCR purification kit (Qiagen Inc., USA) and sequenced by a dideoxy termination with an automatic sequencer (ABI PRISM® 3700 DNA Analyzer, Applied Biosystems, USA). The sequence data were collected using ABI Prism Data Collection software (ver. 2.1), and analyzed by ABI Prism Sequence

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Table 1. BVDV cases sequenced in this study from Republic of Korea

Sample ID[a]	Age (days)	Sex	Clinical Signs	Genotype
1	240	M	Respiratory disorders /Diarrhea	2a
2	180	F	Respiratory disorders /Diarrhea	2a
3	240	F	Respiratory disorders	2a
4	150	F	Respiratory disorders	2a
5	270	F	Respiratory disorders	2a
6	240	F	Respiratory disorders	2a
7	240	F	Respiratory disorders	2a
8	150	M	Respiratory disorders /Diarrhea	2a
9	120	M	Respiratory disorders /Diarrhea	2a
10	35	M	Respiratory disorders /Diarrhea	2a
11	102	M	Respiratory disorders /Diarrhea	2a
12	150	M	Respiratory disorders /Diarrhea	2a

analysis software (ver. 2.1.1) and Chromas software (ver. 1.51) (Technelysium Pty Ltd., Australia). The nucleotide sequences were aligned initially using the ClustalX (1.60) (13). Aligned sequences were examined with a similarity matrix. Relationships between individuals were assessed by neighbor-joining (NJ) method with nucleotide distances (*p*-distance) with 100 replications in the bootstrap test. Phylogenetic analyses based on the obtained sequences were conducted using maximum-likelihood (12).

The GenBank accession numbers of BVDV 5'-UTR sequences used to construct the phylogenetic tree were: CH470, Chile (AY 671984); 890, USA (U18059); KZ-91-CP, Japan (AB003619); KZ-91-NCP, Japn (AB003620); OY89, Japan (AB003621); SW-90, Japan (AB003622); MS-1, Japan (AB019688); 1494, Canada (L32893); BVDV7937, USA (AF039175); CH113, Chile, (AY671978); Bernenices, USA (FJ387269); Columbia, USA (FJ387274); Venus, USA (FJ387309).

Discussion

BVDV infection was performed by RT-PCR using nasal discharges and their expected fragment was 288 bp in length. Twelve of 21 cattle (57%) were identified as positive for BVDV and classified as PI. The median age of these PI cattle was 176 days, i.e. six months, and the age difference between youngest and oldest PI animal varied between 35 and 270 days (Table 1). They had experienced clinical signs such as respiratory disorders with coughing and nasal discharges, and diarrhea. This nasal swab sampling used in this study was easy method, and can be performed without any special technique and equipment. Therefore, this observation indicated that nasal swab could be useful for the detection of PI cattle by RT-PCR and PI cattle might spread the virus in the herd through nasal discharge.

All of the amplified 5'-UTR from 12 PI cattle were exactly identical among amplicons and deposited to GenBank as N1-KOR (GenBank accession number, GQ360074). Our case was closely related to Chile, displaying 99% nucleotide sequence identity (data not shown). To understand a genetic relationship, a phylogenetic analysis was performed on the basis of the 5'-UTR sequences of 14 BVDV isolates worldwide, including our case sequenced in this study. The phylogenetic analysis revealed that our BVDV field case belonged to BVDV-2a subgroup, and our case was more related to Chile and USA isolates, which were highly pathogenic (Fig 1). It is possible that BVDV-2a is the predominant subgroup circulating in Korean indigenous cattle. However, our neighbor country, Japan BVDV isolates formed a distinguishable branch that is separate from our case, and had been low pathogenic.

The present study described the occurrence of 12 PI cattle using nasal swabs. Although PI cattle play an important role in the epidemiology of BVDV infection, it is difficult to detect PI animals routinely, because PI animals remained undetected and subsequently gave birth to new generation of PI calves (2-4). Therefore, PI cattle contribute to the introduction and continued circulation of BVDV within a herd. As a result, the identification and eradication of PI animals might be important for the prevention of BVDV infection and the reduction of great economic damage to the Korean livestock industry. Thus, continuous surveillance for BVDV PI using nasal swabs might be necessary to avoid transmission and persistence of BVDV.

In conclusion, this finding demonstrated the occurrence of BVDV-2a in Korean indigenous cattle. The majority of the Korean cases to date characterized are related to the same subgroup (7). This study underlines the need for the adequate prevention and control measures. Even more important, the presence of BVDV could represent a more significant threat to cattle health that was previously unknown in Korean livestock industry. In Korea, there is no BVDV national control program, and the management practices, such as cattle trade and movement, expose cattle herds to a high risk of introduction of BVDV-2a infection. This result may influence the type strain used in the vaccine and vaccination protocols. Further research should focus on the characterization of more BVDV isolates from different regions of Republic of Korea.

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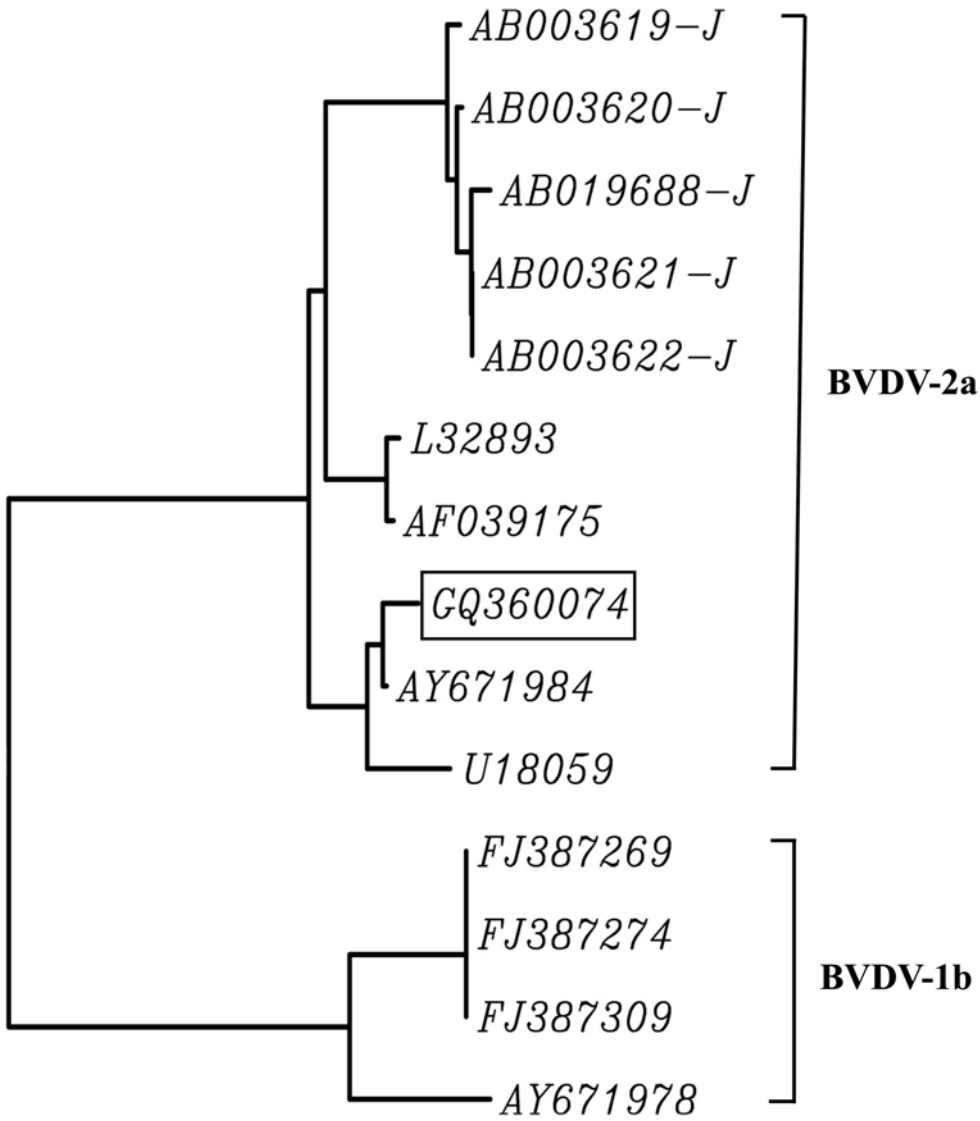

Fig 1. Phylogenetic analysis of BVDV PI isolates based on the 5'-UTR sequences. An unrooted neighbor-joining tree was constructed from 14 genome sequences. Bootstrap values are indicated as a percentage for 100 replicates. The genotype is shown in the right part of the corresponding grouping. Our case sequenced in this study is indicated in a box. J is Japan isolate.

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한국에서 지속감염우의 콧물로부터 소 바이러스성 설사병 바이러스의 계통발생분석

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요 약 : 소 바이러스성 설사병 바이러스는 전세계 축산업에서 경제적 파급력이 큰 질병이다. 콧물을 이용한 역전사 중합효소연쇄반응을 이용하여 한우송아지에서 소 바이러스성 설사병 바이러스의 발생이 진단 되었다. 가검물을 채취한 21 마리 소 가운데 12마리가 소 바이러스성 설사병 바이러스에 양성반응을 보여 지속감염우로 분류되었다. 이 동물들은 설사와 폐렴 같은 증상을 나타내었다. 지속감염우는 일발적으로 지속감염 송아지에서 발견된다. 5'-UTR을 이용한 염기서열 및 계통발생 분석에서 본 증례는 BDVD-2a에 속했다. 이들 결과는 콧물을 이용하여 지속감염우를 진단할 수 있다는 것과, 한우 송아지에서 BVDV 근절 대책이 필요하다는 것을 말해 주고 있다.

주요어 : 소 바이러스성 설사병 바이러스; 콧물; 지속감염우; BVDV-2a.