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Pathobiological Analysis of Bovine Viral Diarrhea Virus Identified in the Republic of Korea

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Abstract : Phylogenetic and nucleotide analysis revealed that severe acute bovine viral diarrhea virus (BVDV) outbreaks from Korean indigenous calves belonged to BVDV-2a, and were identical to those of the highly pathogenic BVDV-2 strain 890, with identical virulence markers and classified as highly virulent. These outbreaks affected young calves (20 and 40 day-old) and were characterized by hemorrhagic diarrhea, depression, anorexia, and weakness. The identification of the highly virulent BVDV-2 isolates among Korean indigenous calves may have important implications for epidemiological studies, diagnostic and immunization strategies.

Key words **:** bovine viral diarrhea virus, phylogenetic and nucleotide analysis, virulence.

Introduction

Bovine viral diarrhea virus (BVDV) is distributed worldwide in cattle herds and leads to a significant economic impact on cattle producers. BVDV infections have been associated with a variety of clinical manifestations from unapparent or mild to severe acute and fatal mucosal disease (1,4). BVDV has been segregated into two genotypes, BVDV-1 and BVDV-2 (15,17). BVDV-1 usually causes only subclinical symptoms, whereas BVDV-2 not only produces similar clinical signs as BVDV-1, but also outbreaks of acute BVD/ hemorrhagic disease (2,15,17).

Recently, severe outbreaks of acute BVD with death in all age groups have been reported primarily in North America (2,6,10). The BVDV isolates from these outbreaks have been associated with highly virulent strains of BVDV-2 and were genetically different from the classical BVDV strains. Acute disease caused by virulent strains of BVDV-2 is characterized by fever, diarrhea, high abortion, severe thrombocytopenia and fatal hemorrhagic syndrome with high mortality (4).

The BVDV infection is widely spread among Korean indigenous cattle from different regions of the country (14,19). In this study, we report the genetic and pathobiological features of BVDV-2 isolates recently identified in the Republic of Korea (ROK).

Materials and Methods

Clinical outbreaks

Five outbreaks from 5 different farms in Youngju city of Gyeongbuk province, ROK were presented. The outbreaks occurred in the winter of 2010 and only calves between 20 and 40 days old were affected. Clinical signs were characterized by depression, anorexia, hemorrhagic diarrhea, weakness, and respiratory disorders. These farms had no closed herds and a recent introduction of cattle. The diarrhea stools were collected from 5 Korean indigenous calves. The collected samples were immediately frozen at −80°C until used. To investigate the presence of concurrent bacterial infections, diarrheal samples were submitted for bacterial isolation.

RT-PCR and nucleotide sequence

Total RNA was extracted from the diarrhea stools using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcription-polymerase chain reaction (RT-PCR) was performed as previously described (3). Amplification and sequencing of 5'-UTR was carried out using the primers 324 and 326 (23). 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 a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster, CA, USA) and analyzed on an ABI PRISM® DNA analyzer (Applied Biosystems).

Phylogenetic analysis

The obtained sequences data were aligned initially using the Clustal X program (version 1.8) (21). Phylogenetic tree based on the nucleotide alignments was constructed using the neighbor-joining (NJ) method. Bootstrap analysis was carried out 1000 replicates and the tree was visualized using Treeview (16).

Nucleotide accession numbers

The nucleotide sequences of the followings 8 strains were used BVDV-2a; 23025 (AF039172), 17583-97 (AF039176),

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NY93 (AF039173), 890 (U18059), 713-2 (AF039177), 5521-95 (AF039174), 17011-96 (AF039179), and 7937 (AF039175).

Results

These five outbreaks were tested and confirmed positive for BVDV by RT-PCR. The clinical signs from these positive cases were recorded in Table 1. These calves were in acute phase infection and exhibited severe diarrhea. These acutely infected calves showed a fever and were dead within 24 h - 3 days after the first manifestation of infection was observed. All cases occurred in less than 40 days old. Sequencing homology of the BVDV obtained from these outbreaks showed 99.0 - 99.7% nucleotide sequence identity.

A phylogenetic analysis based on the 5'- UTR sequences revealed that the BVDV field cases obtained from these acutely infected animals was identified as BVDV-2a (Fig 1). The correlation between virulence and pathogenicity was investigated. As virulence markers of BVDV-2a, the 215th nucleotide, thymine, and the 274th nucleotide, cytosine, in the 5'-UTR of BVDV-2 strain 890 were reported (22). A sequence alignment of this region among 8 reference strains and 5 our cases was shown in Fig 2. Our cases were identical to those of the highly pathogenic 890, with identical virulence markers (Fig 2), and other 3 isolates (23025, 17583-97, and NY93) were the same as those of ours, demonstrating that these isolates were shown as highly virulent (HV). Four isolates (713-2, 5521-95, 17011-96, and 7937) were related with low virulence (LV), indicating that the sequence alignments of this region were cytosine and thymine, respectively. Calves isolated from severe acute outbreaks in this study were identified with highly virulent BVDV-2a.

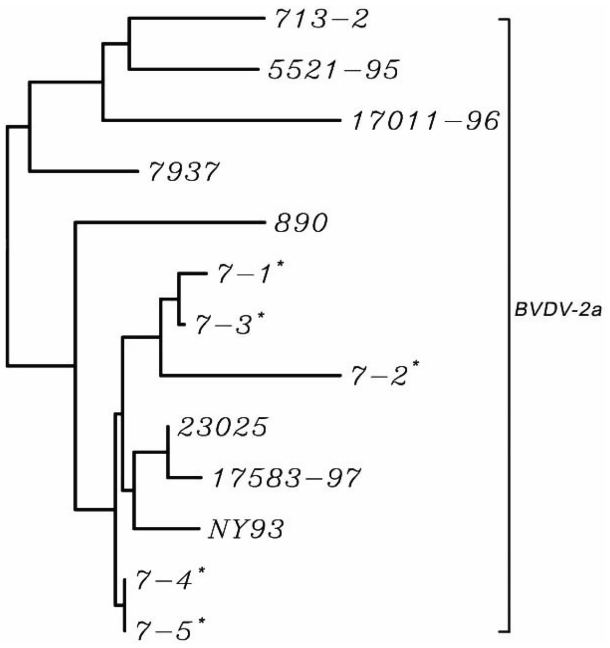

Fig 1. Phylogenetic analysis of BVDV-2 isolates based on the 5'-UTR sequences. An unrooted neighbor-joining tree was constructed from 13 genome sequences. Bootstrap values are indicated as a percentage for 1,000 replicates. Genotype is shown in the right part of the corresponding grouping. Our cases sequenced in this study are indicated by an asterisk.

Fig 2. Comparative analysis of BVDV-2a isolates reported in this study. Two nucleotides (box) are the virulence markers of highly pathogenic BVDV-2a isolates. Nucleotide numbers on the virulence markers originate from the sequence of strain 890. HV is for high virulent isolates and LV is for low virulent isolates.

Table 1. Clinical presentation of calves affected with severe acute outbreaks

Sample ID	Age (days)	Sex	Presentation
7-1	20	M	Hemorrhagic diarrhea, Respiratory disorder, Depression, Anorexia
7-2	25	F	Hemorrhagic diarrhea, Depression, Anorexia
7-3	29	M	Hemorrhagic diarrhea, Depression, Anorexia
7-4	21	M	Hemorrhagic diarrhea, Depression, Anorexia
7-5	38	M	Hemorrhagic diarrhea, Respiratory disorder, Anorexia, Weight loss

Discussion

This finding indicates that these BVDV outbreaks have occurred due to infections with severe hemorrhagic diarrhea. By phylogenetic analysis, the present 5 cases were identified as BVDV-2a. The recent BVDV outbreak in the ROK has been found to be increased in Korea indigenous cattle. Our observations that BVDV-2a is prevalent in the ROK show the same tendency with the previous epidemiological study (19). This result showed that severe acute BVD with hemorrhages were more of the BVDV-2 than BVDV-1 genotype. These results suggest that the BVDV-2a genotype might be circulating for a long time in Korean indigenous cattle.

Identified in outbreaks of severe disease, BVDV-2 was initially thought to be an invariably virulent virus. Further studies demonstrated that BVDV-2 had been circulating in North America for a long time (2,4). Nowadays, these viruses have been identified in several European countries, South America, Japan, and the ROK (3,5,8,9,11-13,20,24). BVDV-2 infections cause the hemorrhagic syndrome in cattle, which can be associated with a high mortality and are presently leading to great economic losses in the ROK. BVDV-2 is associated with severe hemorrhagic disease (18); however, not all BVDV-2 causes clinically severe diseases and only minor fractions of all BVDV-2 isolates are highly virulent (7,17). These results support the fact that the association of BVDV-2 with severe disease indicates the presence of highly virulent isolates in the ROK.

Recently, the emergence of highly virulent BVDV strains has shown the existence of pathogenic differences between BVDV strains. Topliff and Kelling (1998) have reported that the level of virulence is associated with a phylogenic separation of BVDV-2 (22). As shown in Fig 2, two nucleotide substitutions were identified in the internal ribosomal entry site (IRES) that distinguished the high virulence from the low virulence BVDV-2 genotype. Therefore, the substituted bases are virulence markers that were used to identify BVDV-2 of high virulence. In this study, the nucleotide sequences of the present cases in the ROK were almost identical to those of the highly pathogenic BVDV-2 stain 890, with identical virulence markers (Fig 2). These outbreaks are located in the same cluster from strain 890 and other hemorrhagic strains of USA. Our cases had a 96 - 97% homology with strain 890. These outbreaks had high nucleotide identities with the highly pathogenic strain in the 5'- UTR where the viral RNA genome constitutes the IRES that initiates translation. Therefore, further study will be necessary to investigate the different regional variations of BVDV and its association with the virulence of local isolates.

In conclusion, these results indicate that the relationships between genotypes and pathogenicity, or individual status of calves (age and symptoms) were found. The present study suggests that the existence of high virulent BVDV-2 appears to be very high and as such, is at a high risk for the importation of BVDV. This information should be considered to develop diagnostic methods, the need for formulation of vaccines for protection of highly virulent isolates and the design and implementation of biosecurity programs within the Korean livestock industry.

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한국에서 분리된 소 바이러스성 설사 바이러스의 병리생물학적 분석

최경성

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요 약 : 계통발생 및 염기서열분석시 한우 송아지에서 중증의 급성 소 바이러스성 설사 바이러스 발생은 BVDV-2a에 속한 것으로 나타났고, BVDV-2 균주 890과는 독성 마커가 동일하였으며, 독성이 높은 것으로 분류되었다. 이들 BVDV 발생은 어린 송아지(20-40일령)에 주로 감염되었고, 임상증상으로 출혈성 설사, 침울, 식욕감퇴, 허약을 특징으로 하였다. 한우 송아지에서 고독성 BVDV-2의 발견은 역학조사, 진단 및 예방접종 전략에 중요한 영향을 내포한다.

주요어 : 소 바이러스성 설사 바이러스, 계통발생 및 염기서열분석, 독성