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Genetic Characterization of Bovine Viral Diarrhea Virus from Korean Indigenous Calves in Gyeongbuk Province

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Abstract : Bovine viral diarrhea virus (BVDV) is an important worldwide disease in the livestock industry. To characterize BVDV circulating in Gyeongbuk province in the Republic of Korea which has the highest cattle population density, 365 rectal swabs from clinically BVDV Korean indigenous calves were collected. Fifty cases were identified as positive for BVDV. A phylogenetic analysis of 5'- untranslated regions (UTR) revealed that most of our cases belonged to BVDV-2a ($n = 48$), while only two cases were classified as BVDV-1a ($n = 1$) and 1b ($n = 1$), respectively. These results indicated that BVDV-2a is the most prevalent subgroup in Korean indigenous calves of Gyeongbuk province.

Key words **:** Bovine viral diarrhea virus; Korean indigenous calves, phylogenetic analysis; BVDV-2a.

Introduction

Bovine viral diarrhea virus (BVDV) is an economically important worldwide disease and one of the most significant viral pathogens of cattle. BVDV infections cause clinical manifestations characterized by transient fever, leukopenia, diarrhea, respiratory disorder, decreased milk production, severe hemorrhagic syndrome, abortion, congenital defects, and birth of weak or persistently delivery of infected (7,15). Persistently infectected (PI) cattle are reservoirs of infection, and can produce immunotolerant calves that shed the virus in most all secretions throughout their lifetime, infecting herds (6,22). Superinfections of PI animals develop the fatal mucosal disease (MD), which is characterized by fatal watery-bloody diarrhea and ulcerations of the intestinal tract (9,19).

Based on the sequence comparison of the highly conserved 5'-untranslated region (UTR), BVDV can be divided into two genotypes: BVDV-1 and BVDV-2. Recent report has suggested the presence of 13 distinct subgroups within BVDV-1 and at least 2 subgroups of within BVDV-2 (3). While BVDV-1 is widely spread all of the world and associated with mild clinical signs, BVDV2 was first reported in North America as an emergent highly pathogenic viral genotype (4,11,14,16) and recently sporadically detected in Japan, South America, and many European countries (5,8,13,18,23,24).

This study was performed to investigate the prevalence of BVDV cases from Gyeongbuk province, the highest cattle population density in the Republic of Korea (ROK), and to report the genetic diversity of Korean BVDV cases circulating in Korean indigenous cattle.

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Materials and Methods

Clinical samples

The rectal swabs were collected from 365 Korean indigenous calves from 125 different cattle breeding herds belonging to 11 different cities of Gyeongbuk province in the Republic of Korea during 2008 and 2009. The tested animals were aged up to 6 months after birth, and had a history with diarrhea. These animals had no record of vaccination against BVDV. These farms had no history of BVDV detection in the past 1 year. The rectal swabs were immediately frozen at -80°C until analyzed.

RT-PCR

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) from rectal swabs. For RNA extraction, rectal swabs were made into 50% suspensions (v/v) using PBS. RT-PCR reaction was performed with AcessQuick™ RT-PCR System (Promega, Madison, USA) according to the instructions of the manufacturer. Amplification of 5'-UTR was carried out using 324 and 326 primers as previously described (21). The predicted size of the amplified PCR product was 288 bp. Reverse transcription was performed at 45°C for 45 min, and followed by 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. Amplified products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide.

Sequencing and phylogenetic analysis

The PCR products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, USA). The nucleotide sequences were determined by direct sequencing of the PCR products using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster, USA) and analyzed on an ABI

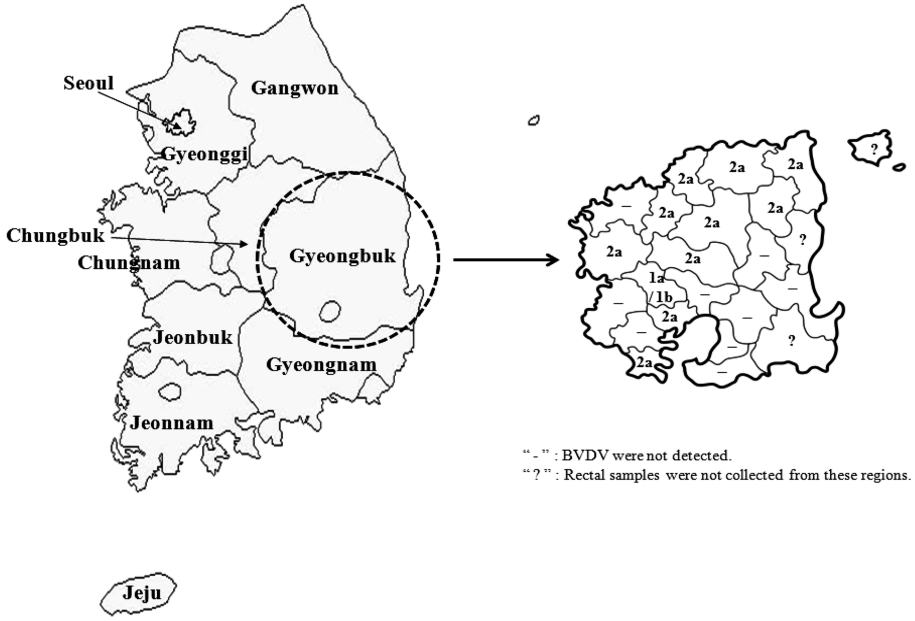

Fig 1. Map of Gyeongbuk province showing the geographical origin of BVDV sequences analyzed in this study.

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

Sample ID	Collection date	Genotype	Herd origin	GenBank Accession No.	Sample ID	Collection date	Genotype	Herd origin	GenBank Accession No.
1-26	Jun/11/2008	BVDV-2a	Andong	GQ161921	4-2	Mar/30/2009	BVDV-1b	Kumi	GQ985459
1-52	Jul/10/2008	"	"	"	4-81	May/21/2009	BVDV-1a	"	GQ985460
2-45	Jan/9/2009	"	"	"	2-1	Nov/12/2008	BVDV-2a	Sangju	GQ985458
2-58[a]	Jan/23/2009	"	"	"	2-2	"	"	"	"
3-14	Feb/20/2009	"	"	"	2-28	Dec/22/2008	"	"	"
3-21	"	"	"	"	2-29	Dec/26/2008	"	"	"
3-23	Feb/22/2009	"	"	"	2-30	Dec/28/2009	"	"	"
2-19	Nov/24/2008	"	Bongwha	GQ985457	4-71	Apr/15/2009	"	"	"
2-20	Nov/29/2008	"	"	"	5-65[a]	Jun/26/2009	"	Sangju	GQ985458
2-22	Dec/8/2008	"	"	"	5-80	Jun/11/2009	"	Yecheon	"
2-24	Dec/15/2008	"	"	"	5-81	Jun/15/2009	"	"	"
2-25	Dec/18/2008	"	"	"	5-90	Jul/3/2009	"		
2-26	"	"	"	"	5-94	Jul/5/2009	"		
2-27	Dec/22/2008	"	"	"	1-12	May/31/2008	"	Youngju	GQ161919
2-11	Nov/23/2008	"	Chilgok	GQ161921	1-22[a]	Jun/10/2008	"	"	"
2-12	"	"	"	"	5-16	May/25/2009	"		
2-14	"	"	"	"	5-42	Jun/10/2009	"	"	"
2-6	Nov/22/2008	"	Euseong	GQ161920	5-87	Jul/14/2009	"	"	"
2-23	Dec/15/2008	"	"	"	2-72	Feb/2/2009	"	Youngyang	"
2-70	Feb/2/2009	"	"	"	3-9	Feb/19/2009	"	"	"
2-71	"	"	"	"	3-10	"	"	"	"
3-32	Mar/5/2009	"	"	"	2-15	Dec/8/2008	"	Uljin	"
3-41	Mar/24/2009	"	"	"	2-16	Nov/14/2008	"	"	"
3-42	"	"	"	"	2-17	Nov/20/2008	"	"	"
5-53	Jun/14/2009	"	"	"	2-18	"	"	"	"

[a]These calves were dead with severe acute BVDV outbreaks.

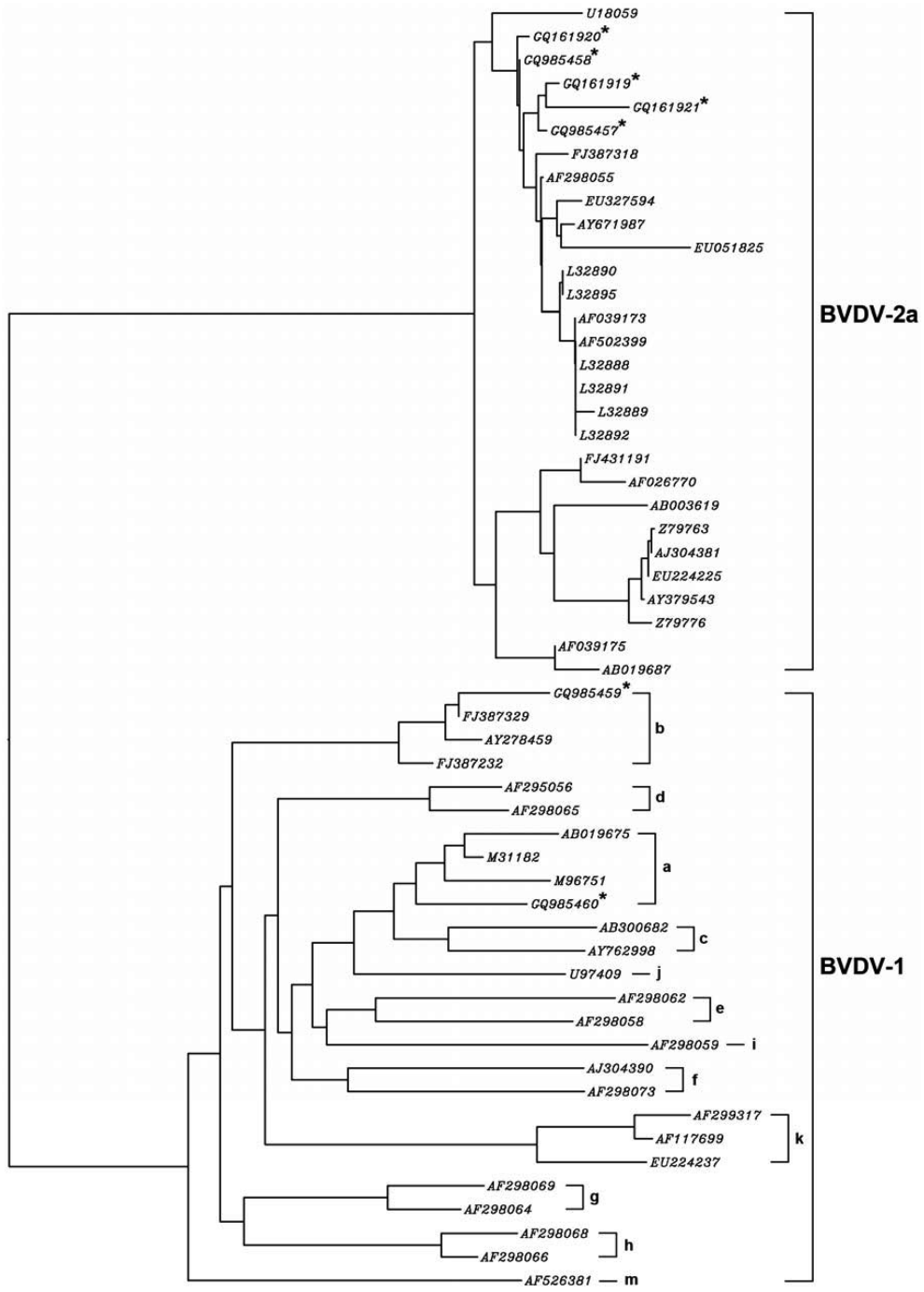

Fig 2. Phylogenetic analysis of the 5'-UTR sequences. The tree was prepared using the neighbor-joining method. Bootstrap values are indicated as a percentage for 1,000 replicates. Each genotype is shown in the right part of the corresponding grouping. Our cases sequenced in this study are indicated in an asterisk.

PRISM® DNA analyzer (Applied Biosystems). The sequences data were aligned initially using the Clustal X (version 1.6) (20). Additional sequences from representative isolates of previously identified BVDV-1 and BVDV-2 were obtained from GenBank and included with each set of alignments. Phylogenetic analysis was constructed using the neighbor-joining (NJ) method with 1,000 replications in the bootstrap analysis. Displaying tree was drawn with the Treeview program (12).

Results

BVDV infection was performed by RT-PCR using rectal swabs. Fifty of 365 samples collected from clinically affected

calves (14%) were identified as positive for BVDV. A survey of the genetic diversity of BVDV field cases in Gyeongbuk province was conducted by determining the nucleotide sequences of the 5'-UTR (Fig 1). On one farm, even two different subgroups occurred. Fig 1 shows the distribution of the distinct subgroups in Gyeongbuk provinces. The pairwise comparison revealed the presence of BVDV-1 ($n = 2$) and BVDV-2 ($n = 48$) (Table 1). Of the 48 cases classified as BVDV-2a, three calves died due to severe acute BVDV (Table 1). The sequence data for the examined BVDV cases have been deposited in the GenBank database with the accession numbers GQ161919 to GQ161921 and GQ985457 to GQ985460.

Nucleotide homology among field BVDV-2a cases ranged from 98.0 to 99.7 %. The complete nucleotide identity was only found among animals of the same herd, in the same area. Due to the large number of samples, a phylogenetic tree was built with only selected isolates. Phylogenetic analysis indicated that most of the BVDV field cases examined in this study was BVDV-2a genotype and two cases assigned to BVDV-1 were subdivided into two subgroups, BVDV-1a and 1b (Fig 2). The Korean BVDV-1a case, GQ985460 and BVDV-1b, GQ985459 had the highest sequence homology against North American reference strains NADL (98.0%) and NY-1 (99.0%) at the 5'-UTR (Fig 2), respectively.

Discussion

In the present study, most cases from Gyeongbuk province were classified as BVDV-2a, whereas BVDV-1a and 1b were isolated only in two out of 50 cases. Our BVDV-1 cases were more related to North America strains. Interestingly, our BVDV-2 cases showed micro-heterogeneity with neighbor country, Japan isolates (AB019687 and AB003619) and were very similar to the highly pathogenic strain, 890 (U18059, USA). Sequence analysis indicated that BVDV-2 is much more prevalent than BVDV-1 in Korean indigenous calves. These results are different from reports from Europe, Argentina, and Brazil in that these countries have reported that only a low amount of BVDV infections are due to BVDV-2 (1,5,8, 22,24). This probably means that there is no correlation between genetic and geographical origin.

BVDV-2 strains in North America were isolated from cattle with clinical signs of hemorrhagic syndrome, which could be associated to high mortality and great economic losses (4,10,14). According to our observations, Korean indigenous calves from which BVDV-2a was isolated exhibited clinical signs of watery diarrhea (88%) and bloody diarrhea (12%) (data not shown). However, our cases (88%) did not show any symptoms of a hemorrhagic syndrome. The relationship between the genotype and clinical symptoms were not found in this study. BVDV-2 outbreak from Korean indigenous calves could explain the failure of vaccines to protect against all the field cases. In general, the predominant BVDV type used in vaccine is BVDV-1a, which is insufficient to block BVDV-2 infection and to prevent the placental infection (2,17). Therefore, our results suggested that a vaccine development, such as the use of vaccine containing both BVDV-1 and BVDV-2 strains, and immunization strategies are recommended for the effective control of BVDV infection in the Republic of Korea.

In conclusion, this study on the genetic characterization of Korean BVDV cases demonstrated that BVDV-2a is predominant in Gyeongbuk province. Only two cases analyzed in this study belonged to BVDV-1a and 1b, respectively. Although these data are not enough on distribution of BVDV, the prevalence of BVDV-1 compared to BVDV-2 might be considered to be very low in Gyeongbuk provice. Paton *et al* (1995) (13) reported that characteristic BVDV isolates were circulating in a particular region and environment. Therefore, further study should be described the genetic characterization whether different regions belong to another genetic group, and focus on BVDV control procedures.

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경북지방에서 사육되고 있는 한우에서 소 바이러스성 설사 바이러스의 유전적 특징

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요 약 : 소 바이러스성 설사 바이러스는 전세계 축산업에서 중요한 질병이다. 대한민국의 최대 소 사육지역인 경북지방에서 유행하고 있는 소 바이러스 설사 바이러스를 조사하기 위해, 소 바이러스성 설사 바이러스의 임상증상을 보이는 한우 송아지로부터 365개의 분변을 채취하였다. 가검물을 채취한 365 마리 송아지 가운데 50 마리가 소 바이러스성 설사 바이러스에 양성반응을 보였다. 5'-UTR을 이용한 계통발생 분석에서 48두 송아지가 BDVD-2a에 속했고, 2두에서만 각각 BVDV-1a와 BVDV-1b로 분류되었다. 이들 결과는 BVDV-2a가 경북지역의 한우 송아지에서 유행하고 있음을 제시하고 있다.

주요어 : 소 바이러스성 설사 바이러스; 한우 송아지; 계통발생분석; BVDV-2a