J Vet Clin 29(5) : 395-399 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

Acute BVDV-1b Outbreak in Korean Indigenous Calves

Kyoung-Seong Choi[1]

School of Animal Science and Biotechnology, College of Ecology and Environmental Sciences, Kyungpook National University, Sangju 742-711, Korea

(Accepted: October 11, 2012)

Abstract : In 2011, several herds in Youngju city in Gyeongbuk province underwent an outbreak of bovine viral diarrhea virus (BVDV) causing high morbidity and mortality. Genetic analysis revealed that two subgenotypes of BVDV-1b (n = 21) and BVDV-2a (n = 7) were identified. The BVDV-1b subgenotype was most frequently detected from our field cases and BVDV-2a subgenotype was also identified in this outbreak. These BVDV-1b infections showed severe acute clinical manifestations similar to BVDV-2 infection. This result reports the detection of BVDV-1b associated with an acute and fatal outbreak of BVDV in Korean indigenous calves.

Key words **:** bovine viral diarrhea virus, BVDV-1b, BVDV-2a.

Introduction

Bovine viral diarrhea virus (BVDV) is the infectious agent of bovine viral diarrhea disease and leads to a substantial economic impact on cattle populations worldwide. BVDV causes a wide variety of diseases including gastrointestinal, hematopoietic, respiratory and reproductive problems. The clinical signs of BVDV infection range from asymptomatic to severe acute disease characterized by hemorrhage syndrome, and fatal mucosal disease in some hypervirulent strains. BVDV is divided into two distinct species, namely BVDV-1 and BVDV-2. For both species, viruses are termed cytopathic (cp) or noncytopathic (ncp) based on the presence or absence of visible cytopathic effects (CPE) in cultured cell (1). Infection of fetuses with ncp BVDV strain during the first trimester of gestation can result in the birth of weak or immunotolerant calves that are persistently infected (PI) (4). These PI calves are a major source of viral spread during their life time.

Recent studies reported that the occurrence of BVDV-1b has increased and caused an acute and fatal outbreak, congenital deformities, acute respiratory disease, and generalized dermatitis (2,6,9,10,15). These BVDV-1b infections showed similar clinical signs to some hypervirulent BVDV-2 strains. Due to similarities in clinical manifestations, the only way to distinguish between these BVDVs is based on molecular analysis. In this study, we describe an acute BVDV-1b outbreak recently detected in the Republic of Korea (ROK).

Materials and Methods

Clinical samples

In the fall of 2011, a total of 203 fecal samples were collected from diarrheic calves in Youngju city which underwent a disease outbreak associated with BVDV infection. The disease progress of these animals was severe, but consisted predominantly of diarrhea, and respiratory symptoms. Some of them were affected with hemorrhagic diarrhea. After birth, the calves started showing diarrhea. Tentative treatment of calves achieved no clinical improvement. Five calves died during this outbreak, but none of these was examined postmortem. The ages of the calves tested ranged from 1 to 30 days. Tests for all other viral and bacterial infections were negative. Information about vaccine history was not provided by the ranchers

RT-PCR

Fecal suspensions of each sample were diluted with phosphate-buffered saline (PBS) and centrifuged at 1,200 × g for 10 min. Viral RNA was extracted from the supernatants using a Trizol (Invitrogen, CA, USA). BVDV was detected using one-step RT-PCR with specific primer sets (324/326) as previously described (4). Amplified products were separated by electrophoresis in 1% agarose gel and visualized by ethidium bromide.

Phylogenetic analysis

The amplified PCR products were purified with a commercial kit (Qiagen Inc., CA, USA) according to the manufacturer's instructions and were sequenced using an ABI PRISM 3700 Genetic Analyzer (Applied Biosystems, CA, USA). The nucleotide sequences were compiled with based on published BVDV sequence information using DNASIS MAX software (Hitachi Solutions Ltd., CA, USA). Phylogenetic analysis was constructed using the neighbor-joining method with MEGA 4.0 software (17). The Evolutionary distances were calculated using Kimura 2-parameter method (8). Bootstrap analysis was carried out using 1,000 replicates and the tree was visualized

[1]Corresponding author.
E-mail : kschoi3@knu.ac.kr

using Treeview (11).

Results

A total of 203 stool samples in 2011 were tested for BVDV and 28 (13.7%) calves were found to be BVDV positive. Genetic analysis revealed that two subgenotypes were identified among these cases: 21/28 (75%) BVDV-1b and 7/28 (25%) BVDV-2a. The BVDV-1b subgenotype was most frequently detected than BVDV-2a in our field cases. The details of these calves classified as BVDV-1b are listed in Table 1. BVDV-1b infected calves exhibited mostly severe diarrhea (15 cases) and respiratory disorder (6 cases), but most of them came from diarrhea. These clinical manifestations and the acute onset of disease were also similar to those observed in severe infection with BVDV-2, but BVDV-1b was classified by phylogenetic analysis. The BVDV isolates in this study, all BVDV-1b, were genetically identical (≥ 99.9%), even though these calves were originated from the different herds. This genotype suggests a common source of virus in the affected herds. Sequencing analysis showed that our field isolate had a sequence similarity from 94.5% and 96%, against BVDV-1b reference strains, Osloss and NY-1, respectively. In a phylogenetic tree, our case was more related to strains/isolates from Canada (Q69 and Q47) and Italy (IT99-4292-2) (Fig 1).

Of seven isolates of classified as BVDV-2a, three cases were shown with severe hemorrhagic diarrhea and four cases had pneumonia (Table 1). All BVDV-2a isolated from seven cases that were sequenced showed 100% homology. The homology of our BVDV-2a isolate was 98% with hypervirulent strain, 890 (U18059). Our BVDV-2a case (2011YJ) formed one group with other Korean isolate (08Q723) (Fig 1). The presenting problem of these farms was that the ranchers purchased all their breeding herds from several sources, and the vaccine history was not provided for these purchased cat-

Table 1. Details of BVDV-1b and BVDV-2a field cases identified in this study

ID	Age (days)	Sex	Origin[a]	BVDV genotypes	Clinical Signs
14	11	M	F1	1b	Respiratory disease
15	2	M	F1	"	Diarrhea, Respiratory disease
22	20	M	F1	"	Diarrhea, death*
17	3	F	F2	"	Dysentery, Dehydration
18	7	F	F3	"	Dysentery, Dehydration
21	20	M	F4	"	Dysentery, Dehydration
23	15	M	F4	"	Dysentery, Dehydration
32	7	M	F5	"	Diarrhea
25	30	M	F6	"	Diarrhea
29	13	F	F6	"	Respiratory disease
30	20	F	F6	"	Diarrhea/Death*
26	17	F	F7	"	Diarrhea
27	12	M	F8	"	Diarrhea
28	10	M	F9	"	Diarrhea, Dehydration
45	21	M	F10	"	Diarrhea, Respiratory disease
70	7	F	F11	"	Diarrhea
71	7	M	F12	"	Respiratory disease
72	30	F	F13	"	Diarrhea
10	2	M	F14	"	Diarrhea
3-14	22	M	F15	"	Respiratory disease
3-21	10	F	F16	"	Hemorrhagic diarrhea, Death*
4-71	10	M	F17	2a	Pneumonia, Diarrhea
5-16	1	M	F18	"	Hemorrhagic diarrhea, Death*
5-42	20	F	F19	"	Hemorrhagic diarrhea
5-87	5	M	F19	"	Pneumonia
6-3	5	F	F19	"	Pneumonia
6-2	10	F	F20	"	Hemorrhagic diarrhea, Death*
6-6	20	F	F21	"	Pneumonia, Diarrhea

[a]The farm (F) number where the case was obtained.
*Death cases

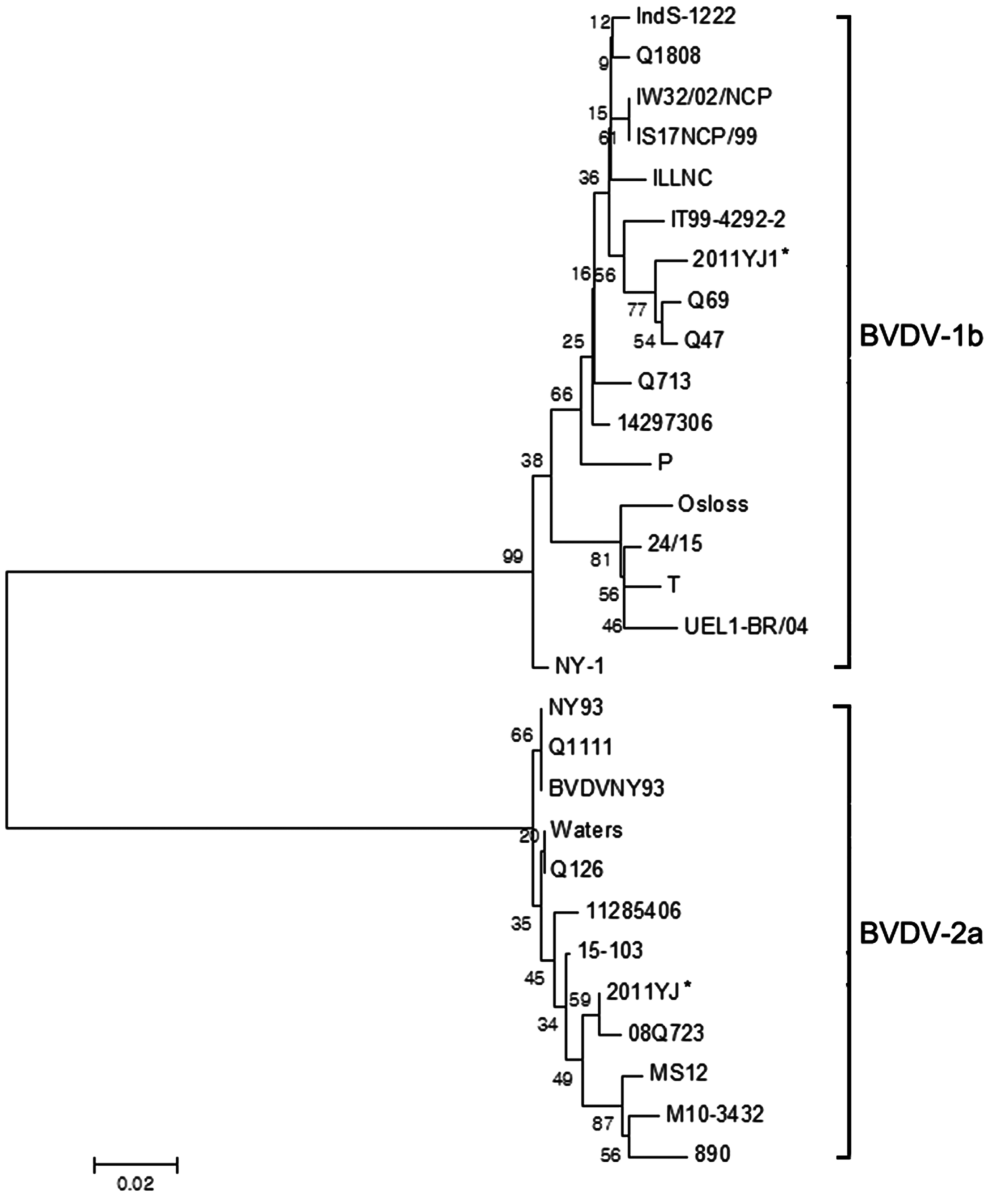

Fig 1. Phylogenetic tree of 5'-UTR sequences using the neighbor-joining method. Bootstrap values for the nodes are shown. Reference strains/isolates are listed in Table 2. Our cases sequenced in this study are indicated in an asterisk.

tle; nor was the cattle tested for BVDV prior to entry into the herds.

Discussion

The present study shows that BVDV-1b is the most prevalent subgenotype identified in our field cases. The clinical manifestations and the acute occurrence presented by these animals were similar to those observed in severe infection with BVDV-2. Although a severe acute outcome of BVD is usually associated with the BVDV-2 strain/isolate, our observation demonstrated that these field infections are caused by BVDV-1b. Previous studies reported that BVDV-1b infections were predominantly involved in inducing respiratory disease in calves and persistent infection (2,3,6). However, the results of this study showed that there was no significant relationship between this subgenotype and particular clinical signs. Due to the timing of the morbidity where identical BVDV-1b isolates were recovered, it appears that the acute BVD infection originated in one farm and then spread to another. The mechanism transmission from farm to farm is unclear as these farms are separated.

The typical clinical signs of BVDV-2 infection were initially thought to be hemorrhagic syndrome or severe acute outbreak (5,7,12,13). However, not all BVDV-2 infections are associated with this severe form (16). Among our seven isolates diagnosed with BVDV-2a, only three cases exhibited such typical clinical signs. The severe acute BVDV-1b infec-

Table 2. Sequences of our cases and the reference strains/ isolates used in this study

Strain/isolate	country	Subgenotype	GenBank Acession No.
2011YJ1	Korea	BVDV-1b	-
Ind S-1222	India	"	AY278459
Q1808	Canada	"	L32884
IW32/02/NCP	Japan	"	AB266477
IS17 NCP/99	Japan	"	AB105770
ILLNC	USA	"	U86600
IT99-4292-2	Italy	"	AJ318599
Q69	Canada	"	L32883
Q47	Canada	"	L32881
Q713	Canada	"	L32882
14297306	USA	"	FJ387329
P	Austria	"	AF298070
Osloss	USA	"	M96687
24/15	UK	"	AF298060
T	Austria	"	AF298072
UEL1-BR/04	Brazil	"	EF406123
NY-1	USA	"	FJ387232
2011YJ	Korea	BVDV-2a	-
890	USA	"	U18059
11285406	USA	"	FJ387318
15-103	France	"	AF298055
Q126	Canada	"	L32890
Waters	Canada	"	L32895
BVDVNY93	USA	"	AF039173
NY93	USA	"	AF502399
Q1111	Canada	"	L32888
O8Q723	Korea	"	JQ418632
MS12	China	"	GU395545
M10-3432	China	"	JN377413

"-" = no submission.

tion was diagnosed as the cause of diarrhea and respiratory symptoms. This data support observations that severe disease is not always related to BVDV-2 and many BVDV-2 infections progress as subclinical forms. Therefore, these findings could not explain the noticeable difference in clinical manifestations between BVDV-1b and BVDV-2a.

Our study indicates that BVDV-1b is the most common subgenotype in this region, and only a few field cases are classified as BVDV-2a. Severe acute outbreaks of BVD are not usually associated with BVDV strains other than BVDV-2, so BVDV-1 infection is normally not related to these outbreaks. However, this result shows the detection of BVDV-1b associated with an acute and fatal outbreak of BVD in Korean indigenous calves. Furthermore, there are currently no diagnostic tests that can definitively distinguish BVDV-1 from BVDV-2. Since molecular characterization is the only method that can identify the BVDV genotype involved in acute outbreaks of severe BVD, it is extremely important to investigate the epidemiological studies and to monitor BVDV infection in cattle herds. In the future, it is necessary to develop the diagnostic system that can separate each subgenotypes between BVDV-1 and BVDV-2.

A recent study demonstrated that BVDV-1b is the prevalent in the United States (14). Due to the widespread use of BVDV-1a based vaccines, the prevalence of BVDV-1a was decreased, while the incidence of BVDV-1b remains unchanged. This suggests that BVDV-1a vaccines uniformly induce little or no immunity to the heterologous BVDV-1b. Therefore, vaccines with BVDV-1a and BVDV-2a should be evaluated for protection against BVDV-1b, and effective BVDV vaccines should be developed and utilized to control this predominant BVDV subgenotype.

In conclusion, this study shows that BVDV-1b is the most prevalent subgenotype in our field cases. These BVDV-1b infections are associated with an acute and fatal outbreak of BVD in Korean indigenous calves. Our data has important implications for BVDV control and also underscores the point that efficacious vaccines controlling BVDV-1b infections are needed. New additions in herds through purchase of pregnant cows should also be investigated. Cows or heifers that test negative could be acutely or transiently infected and may be carrying infected fetuses. Successful control programs should therefore be provided to prevent the introduction of BVDV into herds.

Acknowledgement

This study was supported by Dong-Il Cultural Scholarship Foundation in 2011.

References

1. Baker JC. The clinical manifestations of bovine viral diarrhea infection. Vet Clin North Am Food Anim Pract 1995; 11: 425-445.
2. Blanchard PC, Ridpath JF, Walker JB, Hietala SK. An outbreak of late-term abortions, premature births, and congenital deformities associated with a bovine viral diarrhea virus 1 subtype b that induces thrombocytopenia. J Vet Diagn Invest 2010; 22: 128-131.
3. Baule C, Kulcsar G, Belak K, Albert M, Mittelholzer C, Soos T, Kucsera L, Belak S. Pathogenesis of primary respiratory disease induced by isolates from a new genetic cluster of bovine viral diarrhea virus type I. J Clin Microbiol 2001; 39: 146-153.
4. Choi KS, Song MC. Epidemiological observations of bovine viral diarrhea virus in Korean indigenous calves. Virus Genes 2011; 42: 64-70.
5. Evermann JF, Ridpath JF. Clinical and epidemiologic observations of bovine viral diarrhea virus in the northwestern United States. Vet Microbiol 2002; 89: 129-139.
6. Fulton RW, Ridpath JF, Saliki JT, Briggs RE, Confer AW, Burge LJ, Purdy CW, Loan RW, Duff GC, Payton ME.

Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. Can J Vet Res 2002; 66: 181-190.
7. Kelling CL, Steffen DJ, Topliff CL, Eskridge KM, Donis RO, Higuchi DS. Comparative virulence of isolates of bovine viral diarrhea virus type II in experimentally inoculated six- to nine-month-old calves. Am J Vet Res 2002; 63: 1379-1384.
8. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980; 16: 111-120.
9. Lunardi M, Headley SA, Lisbôa JA, Amude AM, Alfieri AA. Outbreak of acute bovine viral diarrhea in Brazilian beef cattle: clinicopathological findings and molecular characterization of a wild-type BVDV strain subtype 1b. Res Vet Sci 2008; 85: 599-604.
10. Odeon AC, Risatti G, Kaiser GG, Leunda MR, Odriozola E, Campero CM, Donis RO. Bovine viral diarrhea virus genomic associations in mucosal disease, enteritis and generalized dermatitis outbreaks in Argentina. Vet Microbiol 2003; 96: 133-144.
11. Page RD. Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996; 12: 357-358.
12. Pellerin C, van den Hurk J, Lecomte J, Tussen P. Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. Virology 1994; 203: 260-268.
13. Rebhun WC, French TW, Perdrizet JA, Dubovi EJ, Dill SG, Karcher LF. Thrombocytopenia associated with acute bovine virus diarrhea infection in cattle. J Vet Intern Med 1989; 3: 42-46.
14. Ridpath JF, Fulton RW, Kirkland PD, Neill JD. Pre valence and antigenic differences observed between Bovine viral diarrhea virus subgenotypes isolated from cattle in Australia and feedlots in the southwestern United States. J Vet Diagn Invest 2010; 22: 184-191.
15. Ridpath JF, Lovell G, Neill JD, Hairgrove TB, Velayudhan B, Mock R. Change in predominance of Bovine viral diarrhea virus subgenotypes among samples submitted to a diagnostic laboratory over a 20-year time span. J Vet Diagn Invest 2011; 23: 185-193.
16. Song MC, Choi KS. Genetic characterization of bovine viral diarrhea virus from Korean indigenous calves in Gyeongbuk province. J Vet Clin 2010; 27: 220-224.
17. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007; 24: 1596-1599.

한우 송아지에서 급성 BVDV-1b 발생보고

최경성[1]

경북대학교 생태환경대학 축산BT학부

요 약 : 2011년 경북 영주의 여러 농가에서 이병율과 치사율이 높은 소 바이러스성 설사 바이러스(BVDV)가 발생하였다. 유전자 분석 결과 두 개의 유전자형 BVDV-1b (n = 21)와 BVDV-2a (n = 7)이 확인되었다. 검사결과, 이 지역의 농가에서는 BVDV-1b 유전자형이 가장 많이 검출되었고, 또한 몇 농가에서 BVDV-2a 유전자형도 검출되었다. 이들 농가에서 발생한 BVDV-1b 감염은 BVDV-2 감염과 유사한 중증의 급성 임상증상을 보여 주었다. 이 결과는 한우 송아지에서 급성이면서 치명적인 BVDV-1b 발생을 보고한다.

주요어 : 소 바이러스성 설사 바이러스; BVDV-1b; BVDV-2a