J Vet Clin 27(6) : 635-639 (2010)

Journal of Veterinary Clinics
www.ksvc.or.kr

Phylogenetic Analysis of Bovine Viral Diarrhea Virus from Korean Indigenous Calves in Gyeongbuk Province

Moo-Chan Song and Kyoung-Seong Choi[1]

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

(Accepted: November 18, 2010)

Abstract : The prevalence of bovine viral diarrhea virus (BVDV) in Korean indigenous calves with diarrhea in Gyeongbuk province was investigated. Seventy-five cases were identified as BVDV positive in the diarrhea stools. Phylogenetic analysis revealed that all our cases were classified as BVDV-2a. Most of the present BVDV-2a cases were isolated from calves showing clinical signs of watery diarrhea. Our observations indicate that not all BVDV-2 infections cause clinically severe disease. This study shows the high incidence of BVDV-2 infection in Gyeongbuk province. Therefore, the results suggest that a vaccine development and immunization strategies are required for the effective control of BVDV infection in the Republic of Korea.

Key words **:** Bovine viral diarrhea virus, Korean indigenous calves, phylogenetic analysis, vaccine development, immunization strategy.

Introduction

Bovine viral diarrhea virus (BVDV) is one of the most important viral pathogen of cattle that causes fatal diarrhea syndrome, respiratory problems, and reproductive failure. BVDV infections lead to significant economic losses in the livestock industry throughout the world (1,7). Two genotypes of BVDV have been classified, BVDV-1 and BVDV-2 based on the sequence comparison of the highly conserved 5' - untranslated region (UTR) (12,13). BVDV-1 occurs worldwide and is associated with mild clinical signs, whereas BVDV-2 was first identified in North America and causes severe hemorrhagic syndrome with highly pathogenic genotype characterized by fatal acute diarrhea, fatal thrombocytopenia and deaths in all age groups (5,12,13).

The prevalence of the BVDV-2 genotype among Korean indigenous cattle has recently been reported (10,17,18), however clinical and epidemiological studies of BVDV-2 infection in the Republic of Korea (ROK) have not been characterized well, and most BVDV studies focused on the phylogenetic analysis. Therefore, this study was performed to describe the clinical signs and the differences of BVDV outbreak by season and age from Korean indigenous calves within a specific geographic location of the ROK, and to underscore the need for control strategies of BVDV infections in the ROK.

Materials and Methods

Samples

The diarrheal stools were collected from the 473 diseased Korean indigenous calves in Gyeongbuk province. A total of 473 stool samples were provided from 13 different cities of Youngju (190), Andong (27), Bongwha (59), Sangju (20), Gumi (63), Euseong (12), Youngyang (9), Mungyeong (5), Uljin (8), Yeocheon (15), Goryeong (10), Chilgok (53), and Gimcheon (2), respectively (Table 1). The animals were aged up to 6 months after birth, and suffered from diarrhea, respiratory problems, and a loss of appetite. These animals had not been vaccinated for BVDV. Based on anamnesis and clinical signs, these animals were suspected of BVDV infection. No serological tests

Table 1. RT-PCR results for BVDV from Korean indigenous calves (n = 473) with diarrhea in Gyeongbuk province

Herd regions	No. of tested samples	No. of positive samples
Youngju	190	37
Andong	27	2
Bongwha	59	6
Sangju	20	3
Gumi	63	5
Euseong	12	3
Youngyang	9	0
Mungyeong	5	0
Uljin	8	0
Yeocheon	15	5
Goryeong	10	1
Chilgok	53	13
Gimcheon	2	0
Total	473	75

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

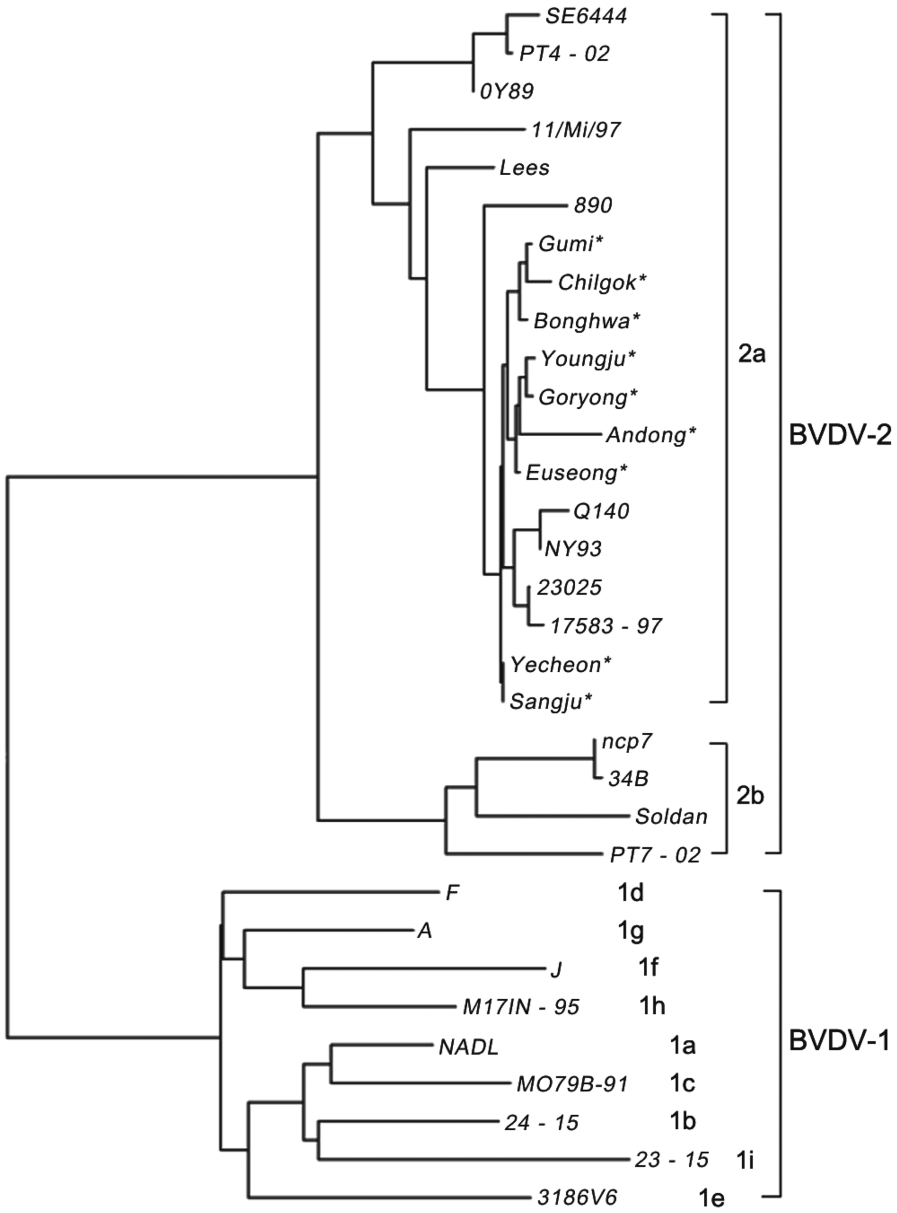

Fig 1. Phylogenetic tree of 5'-UTR nucleotide sequences from Korean BVDV cases and other BVDV strains/isolates. The viral genotypes and subgroups are indicated. The unrooted phylogenetic tree was done by the neighbor-joining methods. Bootstrapping was carried out using 1000 replications. Our cases sequenced in this study are indicated in an asterisk.

were analyzed. BVDV was detected by RT-PCR. To differentiate between BVDV-1 and BVDV-2 genotypes, the positive cases were analyzed at the genetic level.

RT-PCR

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) from the diarrheal stools as described (18). Amplification of 5'-UTR of BVDV was carried out using 324/326 primers (21). The product of amplification was a 288 bp DNA fragment. RT-PCR reaction was performed with AcessQuick™ RT-PCR System (Promega, Madison, USA) according to the instructions of the manufacturer. Reverse transcription was performed at 45ºC for 45 min, and consisted of 30 cycles under following conditions: 94ºC for 30 sec, 55ºC for 30 sec, and 72ºC for 1 min. Amplified products were separated by electrophoresis in 1% agarose gels and visualized by ethidium bromide.

Phylogenetic analysis

The amplified PCR products were purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, USA), directly sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster, USA), and analyzed on an ABI PRISM® DNA analyzer (Applied Biosystems). The alignment of nucleotide sequences was done with the Clustal W program (20). The phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 replications in the bootstrap

analysis. Displaying trees were drawn with the Treeview program (version 1.6) (11). The nucleotide sequences from the representative BVDV-1 and BVDV-2 strains/isolates were included in the phylogenetic analysis. BVDV-1a: NADL (accession number M31182). BVDV-1b: 24-15 (AF298060). BVDV-1c: M079B-91 (U97410). BVDV-1d: F (AF298065). BVDV-1e: 3186V6 (AF298062). BVDV-1f: J (AF298067). BVDV-1g: A (AF298064). BVDV-1h: M17IN-95 (U97431). BVDV-1i: 23-15 (AF298059). BVDV-2a: 17583-97 (AF039176); 23025 (AF039172); NY93 (AF502399); Q140 (L32889); 890 (U18059); OY89 (AB003621); SE6444 (Z79777); PT4-02 (AY944291); Lees (U65051); 11/Mi/97 (AJ293603). BVDV-2b: ncp7 (AY443026); 34B (AF244952); Soldan (U94914); PT7-02 (AY944297).

Results

To determine the prevalence of BVDV infection in Korean indigenous calves with diarrhea, 473 diarrhea stools from 13 different cities in Gyeongbuk province were screened by RT-PCR. Seventy-five out of 473 diarrheic samples (15.9%) were identified as positive for BVDV. The cities and positive numbers of samples for BVDV included Youngju (37), Andong (2), Bongwha (6), Sangju (3), Gumi (5), Euseong (3), Yeocheon (5), Goryeong (1), and Chilgok (13) (Table 1). Genetic analysis of 75 BVDV positive field cases was conducted on the basis of the nucleotide sequence of the 5'-UTR. The phylogenetic analysis revealed that all BVDV field cases examined in this study were classified as BVDV-2a genotype (Fig 1). Many field cases originating from different herds were the same or very similar (98-100%) at the genetic level.

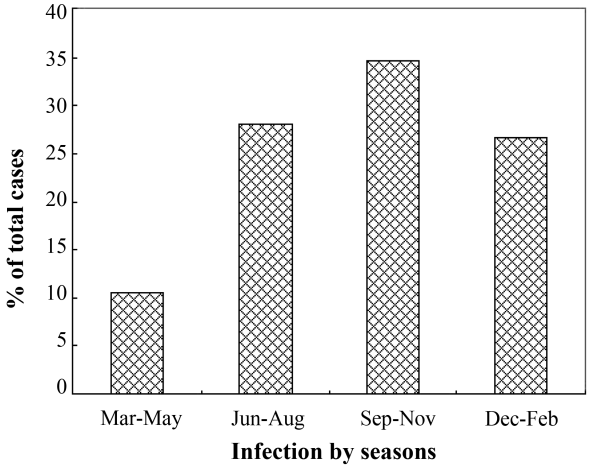

Fig 2. The seasonal changes of BVDV infections from Korean indigenous calves with diarrhea in Gyeongbuk province.

BVDV infections from Korean indigenous calves were investigated by season. Our observation showed that the clinical cases of BVDV were significantly increased in the fall compared to in the other seasons (Fig 2). Seasonally, BVDV infections were as following: 26 out of 75 (34.7%) were positive in the fall, 21 out of 75 (28%) were positive in the summer, 20 out of 75 (26.7%) were positive in the winter and 8 out of 75 (10.6%) were positive in the spring.

When analyzed by age of Korean indigenous calves, most BVDV infections were detected up to 30 days after birth (69.3%), and a significant decrease in infection was observed after 30 days of age, i.e. 60 days (14.7%), 90 days (10.7%), 120 days (2.7%), and 180 days (2.7%) (Table 2). There was no observation of BVDV infection between 120 and 150 days of age. This result showed that the predominant infections were observed in less than 30 days old.

As shown in Table 2, the clinical signs of BVDV-infected Korean indigenous calves ($n = 75$) were as follows: 67 out of 75 (89%) were from watery diarrhea, and 8 out of 75 (11%) were from bloody diarrhea. Five of these calves died due to severe watery diarrhea. Six cases with bloody diarrhea were found in the younger calves < 30 days old (Table 2).

Discussion

In this study, seventy-five cases were detected from 473 Korean indigenous calves by RT-PCR. This represents the first large-scale study of BVDV infection in Korean indigenous calves with diarrhea. Phylogenetic analysis revealed that all cases examined from this survey were classified to BVDV-2a. Interestingly, none of the cases analyzed in this study was belonged to BVDV-1. The result of this analysis demonstrated that BVDV-2a is prevalent among Korean indigenous calves in Gyeongbuk province. It can be concluded that BVDV-1 is either not present or very rare in this region. Therefore, this suggests that there might be one main viral population circulating in this region. The results herein are different from reports from Europe, Brazil, Argentina, South Africa, and Japan that show BVDV-2 has either not been detected or the prevalence is very low with respect to the BVDV-1 genotype (2,6,8,16,22,23). There is now surprising evidence that the recent BVDV-2 outbreak in the ROK was found to have increased in cattle (10, 17,18). It should be pointed out that monitoring of BVDV-2 in the ROK is highly important due to the fact that BVDV-2 infections lead to great economic losses in the livestock industry.

Table 2. Summary of 75 BVDV-positive cases compared with clinical presentation from Korean indigenous calves by age

Clinical presentation	No. of cases	Age (days)					
		0-30	31-60	61-90	91-120	121-150	151-180
Diarrhea-watery	67	46	10	7	2	0	2
Diarrhea-bloody	8	6	1	1	0	0	0
Total	75	52	11	8	2	0	2

There were several important observations that were derived from this study. BVDV infections by season indicated that otherwise BVDV outbreaks were predominant in the winter; our result revealed that BVDV infections occurred most frequently in the fall. To our knowledge, this is the first report showing a clear seasonal distribution of BVDV infections in Korean indigenous calves. Although our observations were significant, its precise cause of the high prevalence of BVDV infections in the fall is unclear. Therefore, further epidemiological studies throughout the ROK are needed to properly understand the seasonal pattern of BVDV infections and to establish BVDV surveillance programs to prevent BVDV infections.

Our study clearly showed that BVDV infection was associated with diarrhea in calves, especially those less than 30 days of age. The comparative analysis of the age profiles might be an important indicator for the changes in the infection rate of younger animals. The high occurrence of BVDV infection at an early age (~30 days) could be attributed to an increased susceptibility of pregnant cattle and the physiologic immune suppression which occurs during pregnancy (9,19). This result reinforces the need for an effective BVDV vaccine that protects the fetus in utero infection.

BVDV-2 infections were generated by the observation that these strains could be associated with hemorrhagic syndrome (HS), a clinically severe form of acute BVDV with high mortality (4,12,13,15). However, the fact that not all BVDV-2 cause HS demonstrated that our cases (89%) did not show any symptoms of a HS (Table 2). A relationship between genotypes and pathogenicity, or individual status of cattle and symptoms could not be established. To clarify the molecular basis of the pathogenicity of BVDV, consecutive monitoring of infections and the determination of virulence factors are required. Thus, BVDV-2 infections might present the clinical symptoms ranging from inapparent to severe in the field. The result of this study suggests that all BVDV-2 isolates do not cause clinically severe disease such as HS, and the severity of disease resulting from BVDV-2 infection could appear to be strain dependent.

The data on incidence of BVDV-2 obtained in this study confirmed that BVDV-2 is one of the major causes of diarrhea in calves. Although these data are not enough on distribution of BVDV infections in the ROK, the prevalence of BVDV-1 compared to BVDV-2 might be considered to be very low in Gyeongbuk province of the ROK. The high incidence of BVDV-2 genotype means that these viruses have been circulating for a long time in cattle in the ROK, which could be explained by the intensive movements of cattle between farms within the same or different regions and by poor BVDV control procedures. These findings have important implications for diagnosis, control, vaccine development and immunization strategies. The BVDV-1a, NADL stain used extensively in the vaccine formulation, predicts the low efficiency of classical BVDV vaccines toward BVDV-2 isolates, raises the question about the degree of protection conferred by commercially available vaccines and may indicate the need for formulation of vaccines based on Korean isolates.

In conclusion, this study demonstrates that BVDV-2a is predominant and widespread in Korean indigenous calves with diarrhea in Gyeongbuk province. In addition, the cases obtained for this study from Korean indigenous calves were found mostly in less than 30 days of age and in the fall. Information obtained from this report will also be useful when carrying out epidemiological surveys of domestic BVDV.

Acknowledgements

This work was supported by the Kyungpook National University Research Grant, 2010.

References

1. Baker JC. Bovine viral diarrhea virus: a review. J Am Vet Med Assoc 1987; 190: 1449-1458.
2. Barros SC, Ramos F, Paupério S, Thompson G, Fevereiro M. Phylogenetic analysis of Portuguese bovine viral diarrhea virus. Virus Res 2006; 118: 192-195.
3. Brock KV, McCarty K, Chase CC, Harland R. Protection against fetal infection with either bovine viral diarrhea virus type 1 or type 2 using a noncytopathic type 1 modified-live virus vaccine. Vet Ther 2006; 7: 27-34.
4. Carman S, van Dreumel T, Ridpath J, Hazlett M, Alves D, Dubovi E, Tremblay R, Bolin S, Godkin A, Anderson N. Severe acute bovine viral diarrhea in Ontario, 1993-1995. J Vet Diagn Invest 1998; 10: 27-35.
5. Corapi WV, Elliott RD, French TW, Arthur DG, Bezek DM, Dubovi EJ. Thrombocytopenia and hemorrhages in veal calves infected with bovine viral diarrhea virus. J Am Vet Med Assoc. 1990; 196: 590-596.
6. Flores EF, Ridpath JF, Weiblen R, Vogel FS, Gil LH. Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. Virus Res 2002; 87: 51-60.
7. Houe H. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Vet Microbiol 1999; 64: 89-107.
8. Jones LR, Zandomeni R, Weber EL. Genetic typing of bovine viral diarrhea virus isolates from Argentina. Vet Microbiol 2001; 81: 367-375.
9. Mellor AL, Munn DH. Extinguishing maternal immune responses during pregnancy: implications for immunosuppression. Semin Immunol 2001; 13: 213-218.
10. Oem JK, Hyun BH, Cha SH, Lee KK, kim SH, Kim HR, Park CK, Joo YS. Phylogenetic analysis and characterization of Korean bovine viral diarrhea viruses. Vet Microbiol 2009; 139: 356-360.
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. Ridpath JF, Bolin SR, Dubovi EJ. Segregation of bovine viral diarrhea virus into genotypes. Virology 1994; 205: 66-74.
14. Ridpath JF, Bolin SR. Differentiation of types 1a, 1b and 2

bovine viral diarrhoea virus (BVDV) by PCR. Mol Cell Probes 1998; 12: 101-106.
15. Ridpath JF, Neil JD, Frey M, Landgraf JG. Phylogenetic, antigenic and clinical characterization of type 2 BVDV from North America. Vet Microbiol 2000; 77: 145-155.
16. Shimazaki T, Sekiguchi H, Nakamura S, Taguchi K, Inoue Y, Satoh M. Segregation of bovine viral diarrhea virus isolated in Japan into genotypes. J Vet Med Sci 1998; 60: 579-583.
17. Song MC, Choi KS. Phylogenetic analysis of Bovine viral diarrhea virus from nasal swab sample of persistently infected cattle in Republic of Korea. J Vet Clin 2009; 26: 582-585.
18. Song MC and Choi KS. Outbreak of Bovine viral diarrhea virus in Korean indigenous calf. J Vet Clin 2009; 26: 578-581.
19. Swasdipan S, Bielefeldt-Ohmann H, Phillips N, Kirkland PD, McGowan MR. Rapid transplacental infection with bovine pestivirus following intranasal inoculation of ewes in early pregnancy. Vet Pathol 2001; 38: 275-280.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22: 4673-4680.
21. Vilcek S, Herring AJ, Herring JA, Nettleton PF, Lowings JP, Paton DJ. Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch Virol 1994; 136: 309-323.
22. Vilcek S, Drew TW, McGoldrick A, Paton DJ. Genetic typing of bovine pestiviruses from England and Wales. Vet Microbiol 1999; 69: 227-237.
23. Wolfmeyer A, Wolf G, Beer M, Strube W, Hehnen HR, Schmeer N, Kaaden OR. Genomic (5'UTR) and serological differences among German BVDV field isolates. Arch Virol 1997; 142: 2049-2057.

경북지역에서 사육되고 있는 한우 송아지에서 소 바이러스성 설사 바이러스의 계통발생 분석

송무찬 · 최경성[1]

경북대학교 생태환경대학 축산학과

요 약 : 경북지역에서 설사증상을 보이는 한우송아지를 대상으로 소 바이러스성 설사 바이러스의 유병률이 조사되었다. 75두의 송아지가 소 바이러스성 설사 바이러스에 양성 반응을 나타내는 것으로 확인되었다. 계통발생 분석시 양성반응을 보이는 75두 송아지가 BVDV-2a로 분류되었다. BVDV-2a로 분류된 대부분은 수양성 설사의 임상증상을 나타내는 송아지로부터 채취한 것이었다. 우리의 결과에 따르면 모든 BVDV-2 감염이 심각한 임상증상을 보이지 않는 것으로 나타났다. 이 연구는 경북지역에서 BVDV-2 감염의 높은 발생률을 보여준 것으로, 이 연구 결과는 백신개발 및 예방 전략이 대한민국에서 BVDV 감염의 효과적인 근절을 위해 필요한 것으로 사료된다.

주요어 : 소 바이러스성 설사 바이러스, 한우 송아지, 계통발생분석, 백신개발, 예방 전략