Biotype, Genotype, and Clinical Presentation Associated With Bovine Viral Diarrhea Virus (BVDV) Isolates From Cattle

Byung Chul Ahn, DVM, PhD[1]
Paul H. Walz, DVM, PhD, DACVIM[2]
George A. Kennedy, DVM, PhD, DACVP[1]
Sanjay Kapil, DVM, PhD, DACVM[1]

[1]*Department of Diagnostic Medicine/Pathobiology*
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas

[2]*Departments of Clinical Sciences and Pathobiology*
College of Veterinary Medicine
Auburn University
Auburn, Alabama

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ABSTRACT

The clinical manifestations that are associated with bovine viral diarrhea virus (BVDV) infection are diverse. In this retrospective analysis of 177 BVDV-positive samples from 1992–2000, we determined the distribution of genotype and biotype. In addition, we compared the relationship among biotype, genotype, clinical sign, and age of animal at time of BVDV isolation, where information was available. Of the 177 BVDV-positive samples, 30% contained cytopathic (CP) isolates and 70% contained non-cytopathic (NCP) isolates. Genotyping using a 5'UTR region-based polymerase chain reaction revealed that 63% were BVDV-1, 26% were BVDV-2, and 11% of the samples contained both BVDV-1 and BVDV-2. Distribution of genotype within each biotype was proportional to the overall distribution for genotype. Isolation frequency by the defined clinical syndrome was highest for respiratory signs (27%), abortion (19%), and diarrhea (18%). The distribution of BVDV genotypes within each clinical syndrome was similar to the overall distribution of genotype, with the exception of oral ulceration. This clinical sign was more frequently reported with BVDV-2 isolation than BVDV-1 isolation. Most BVDV isolates (72%) were recovered from cattle under 12 months of age, the highest frequency (34%) being among calves 4–6 months of age. These results suggest that clinical syndromes are not confined to a specific biotype or genotype, and BVDV virulence may be influenced by factors other than genotype or biotype.

INTRODUCTION

Bovine viral diarrhea virus (BVDV), due to its economic consequences, is an important viral pathogen of cattle worldwide. It can

infect cattle of all ages, with consequences of infection in immunocompetent cattle ranging from subclinical or mild disease to a highly fatal form.[1,2] In addition, BVDV infection of susceptible pregnant cattle may result in fetal infection, with early embryonic death, abortion, or congenital malformations as possible outcomes, as well as the birth of calves immunotolerant to, and persistently infected (PI) with, BVDV. Not only are PI cattle the major source of virus transmission within and among cattle herds, but they are also at risk of developing mucosal disease (MD).[3]

Bovine viral diarrhea virus belongs to the genus *Pestivirus* in the family *Flaviviridae*.[4] Isolates of BVDV can be segregated into 2 biotypes, cytopathic (CP) and non-cytopathic (NCP), based on their effect in cultured cells. Based on their genomic differences, BVDV isolates can be separated into 2 distinct genotypes, BVDV-1 and BVDV-2.[5] Viruses of either genotype may exist as either biotype. The NCP isolates of BVDV-2 have been associated with outbreaks of severe clinical disease in the United States and Canada.[2] These BVDV infections have demonstrated that NCP BVDV can cause severe disease with high mortality in all age groups. In addition, severe thrombocytopenia and a hemorrhagic syndrome also have been associated with infection with NCP BVDV-2.[6,7] Although NCP BVDV-2 may be associated with severe clinical disease, BVDV-2 isolates also may induce subclinical or mild disease.[2,8]

Distribution data of biotype and genotype with clinical presentation is very important not only in controlling and understanding BVDV disease, but also in directing the choices for antigens to be utilized in vaccine development. The purpose of this study was to assess distribution of biotype and genotype for 177 BVDV-positive bovine samples from Kansas. A further objective, reliant upon information availability, was to compare the relationship among biotype, genotype, clinical sign, and age of animal for these BVDV-positive bovine samples.

MATERIALS AND METHODS

Review of Case Records

All case records from BVDV-positive samples from January 1992 to December 2000 were retrieved and reviewed. Where available, the diagnosis and/or clinical presentation, age of the animal, date of submission, and the source of material for virus isolation were recorded.

Virus

One hundred seventy-seven BVDV-positive samples submitted to Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, were evaluated. All samples were classified as positive for BVDV by routine virus isolation procedures performed by Kansas Diagnostic Laboratory, Manhattan, Kansas. The biotype was determined for each BVDV-positive sample according to the effect on cultured embryonic bovine kidney (EBK) cell monolayers, with CP isolates displaying characteristic CP effect on EBK cell monolayers.

Determination of Genotype

During propagation of virus on EBK cells, viral RNA was harvested when approximately 90% CP effect was observed for CP isolates, and at 4 days after inoculation for NCP isolates. Viral RNA was prepared from 140 μL of virus-infected cell culture supernatant using a commercial viral RNA extraction kit (QIAGEN Inc., Valencia, CA). Viruses were genotyped using the 5'UTR region-based polymerase chain reaction (PCR) assay.[5,9–11] All primer sets were synthesized according to previously described primer sequences, with the exception of the BVDV-1 and BVDV-2 common primer set. We designed this primer (forward: 5'GCT AGC CAT GCC CTT AGT AGG ACT 3'; reverse: 5'CAA CTC CAT GTG CCA TGT ACA GCA G 3') using published BVDV sequences.[12–15] For the reverse transcription (RT)-PCR, a 20-μL master mix was made

Table 1. Distribution of Genotype and Biotype From the 177 BVDV-Positive Samples.

	Genotype			
Biotypes	BVDV-1	BVDV-2	BVDV-1 & -2	Total
CP	34	15	4	53
NCP	77	31	16	124
Total	111	46	20	177

Biotype was determined based upon the effect of viral infection on cell culture monolayers, while genotype was determined using a 5'UTR-region-based PCR assay.

containing 9.5 μL of extracted viral RNA, 2 μL of 10× buffer, 1.88 mM of $MgCl_2$, 2.5 μM of primer, 0.5 mM of each deoxyribonucleoside triphosphate (dNTP), 20 U of RNase inhibitor, and 50 U of murine leukemia retrovirus RT (Perkin-Elmer, CA). The RT (first strand synthesis) reaction was incubated at 42˚C for 30 minutes, denatured at 99˚C for 5 minutes, and then quenched at 5˚C for another 5 minutes. After RT reaction, PCR was performed for viral RNA detection. A 30-μL aliquot of master mix containing 3 μL of 10× buffer, 1.66 μM of primer, 0.8 mM of $MgCl_2$, and 1.25 U of AmpliTaq DNA polymerase (Perkin-Elmer, CA) was dispensed into the completed RT reaction tubes. Amplification was performed using 25 cycles under the following conditions: 94˚C for 15 seconds (denaturation), 56˚C for 15 seconds, 72˚C for 30 seconds (25 cycles), and 72˚C for 10 minutes (1 cycle). For the second-round genotype-specific PCR, the 50 μL of reaction included 1 μL of cDNA, 5 μL of 10× buffer, 2 mM of $MgCl_2$, 1 μM of each primer, 0.2 mM of each dNTP, and 1.25 U of AmpliTaq DNA polymerase. The genotyping PCR was performed under the following conditions: 27 cycles, 94˚C for 15 seconds, 49˚C for 15 seconds, 72˚C for 10 seconds, and 1 cycle 72˚C for 10 minutes. The PCR products were separated by agarose gel electrophoresis (1%) and stained with ethidium bromide.

RESULTS

Biotype and Genotype of BVDV Field Isolates

Of the 177 isolations (Table 1), NCP biotypes (70%) were more frequently isolated than CP biotypes (30%). Genotyping revealed that 63% of the BVDV-positive samples contained BVDV-1 isolates, 26% contained BVDV-2 isolates, and 11% of the BVDV-positive samples contained both BVDV-1 and BVDV-2 isolates together. When subgenotyping of the 111 BVDV-1 isolates was performed, 50 isolates were genotyped as BVDV-1a, 58 isolates were genotyped as BVDV-1b. Three isolates could not be subgenotyped, and thus could be non–BVDV-1a or non–BVDV-1b. The distribution of genotype within each biotype was similar to the overall distribution.

Table 2. Clinical Sign and Genotype of BVDV Isolates From Cattle.*

Clinical Sign	BVDV-1	BVDV-2	BVDV-1 & -2
Respiratory	22	9	3
Diarrhea	15	5	2
Abortion	15	7	2
Multiple signs	10	4	3
Oral ulceration	1	4	0
Opaque eye	6	0	0
Nasal hemorrhage	3	2	0
Others†	7	3	3
Total	79	34	13

*Clinical sign information was available for 126 of the 177 BVDV-positive samples.
†Other signs included sudden death, lethargy, neurological signs, wasting, and arthritis.

Clinical Signs, Genotype, and Biotype

Of the 177 BVDV-positive bovine samples, information relating to clinical signs was available for 126 samples (Tables 2 and 3). Clinical signs that were consistent with respiratory disease, diarrhea, abortion, oral ulceration, nasal hemorrhages, sudden death, lethargy, neurological signs, wasting, and arthritis were reported from cattle with BVDV isolation. The most common clinical signs reported included signs relating to the respiratory system (27%), abortion (19%), and diarrhea (18%). When clinical signs were compared with the genotype, BVDV-1 was the predominant genotype in all cases,

Table 3. Clinical Signs and Biotype of BVDV Isolates From Cattle.*

Clinical Signs	CP	NCP
Respiratory	12	22
Diarrhea	13	9
Abortion	5	19
Multiple signs	7	10
Oral ulceration	0	5
Opaque eye	1	5
Nasal hemorrhage	1	4
Others†	2	11
Total	41	85

*Clinical sign information was available for 126 of the 177 BVDV-positive samples.

†Other signs included sudden death, lethargy, neurological signs, wasting, and arthritis.

with the exception of cases exhibiting oral ulceration as the only clinical sign reported (Table 2). The percentages of BVDV-1 were similar in cases with signs referable to the respiratory system (65%) or diarrhea (68%) as the percentage of BVDV-1 for all cases (64%). The percentage of BVDV-2 was higher for abortion (32%), oral ulceration (80%), and nasal hemorrhage (40%) cases than the percentage of BVDV-2 for all cases (27%). In cases with opaque eyes, only BVDV-1 genotype was isolated. When the biotype was compared with clinical signs, NCP biotype was more frequent than CP biotype in all case-categories with the exception of diarrhea. In diarrhea cases, CP accounted for 59% of the cases, and NCP accounted for 41% (Table 3). The percentage of NCP biotype was higher in cases of abortion (79%), oral ulceration (100%), opaque eye (83%), and nasal hemorrhage (80%) than the percentage of NCP BVDV for all cases (68%).

From the BVDV-positive samples, 19 were obtained from aborted fetuses (Table 4). With respect to biotype, 26% contained CP isolates, while 74% contained NCP isolates. When genotyped, 58% and 32% were BVDV-1 and BVDV-2, respectively. The remaining 10% contained both BVDV-1 and BVDV-2. Second trimester fetuses

Table 4. Distribution of Genotype and Biotype From BVDV-Positive Aborted Fetuses.

	BVDV-1		BVDV-2		BVDV-1 & -2	
Age of Fetus	CP	NCP	CP	NCP	NCP	Total
1-3 months	1	0	0	1	0	2
4-6 months	1	7	2	2	2	14
7-9 months	1	1	0	1	0	3
Total	3	8	2	4	2	19

Table 5. Distribution of Genotype and Biotype for BVDV-Positive Samples Where Information on Outcome Was Available.*

	Genotype			
Status	BVDV-1	BVDV-2	BVDV-1 & -2	Total
Alive	28	19	4	51
Dead	62	22	11	95

	Biotype		
Status	CP	NCP	Total
Alive	10	41	51
Dead	34	61	95

*Information related to alive or dead status was available for 146 of the 177 BVDV-positive samples, excluding the cases involving aborted fetuses.

(fetal age, 4–6 months) comprised the majority of the BVDV-positive aborted fetuses (74%), while 11% were obtained from aborted fetuses aged 1–3 months and 16% were obtained from aborted fetuses aged 7–9 months.

Excluding the 19 BVDV isolations from aborted fetuses, 158 BVDV-positive samples were obtained from postnatal cattle. Of these, the alive or dead status of the case at the time of virus isolation was not available for 12 BVDV-positive cases. From the remaining 146 cases where alive/dead status information was known, 95 (60%) were recovered from cattle that had died; 51 (32%) were alive at the time of virus isolation according to the case reports (Table 5). With respect to genotype, the distribution of isolates recovered from cattle that died was 65% BVDV-1, 23% BVDV-2, and 12% BVDV-1 and BVDV-2 together, similar to

Table 6. Clinical Signs and Age of Animal at Time of BVDV Isolation.*

Clinical Signs\Age	1-3 Months	4-6 Months	7-12 Months	Over 1 Year	Total
Respiratory signs	4	15	4	4	27
Diarrhea	1	8	7	4	20
Oral ulceration	0	1	0	2	3
Opaque eye	0	2	0	3	5
Multiple signs	4	6	5	1	16
Nasal hemorrhage	1	3	1	0	5
Others†	8	2	1	1	12
Total	18	37	18	20	93

*Information on age at the time of virus isolation and clinical signs was available for 93 of the 177 BVDV-positive cases.
†Others signs included sudden death, lethargy, neurological sign, wasting, and arthritis.

the overall distribution by genotype. However, the distribution of genotype in cases that were alive at the time of virus isolation was 55% BVDV-1, 38% BVDV-2, and 8% BVDV-1 and BVDV-2 together, different from the overall distribution of genotype by having a higher percentage of BVDV-2 isolations. With respect to biotype, the distribution of isolates recovered from cattle that died was 64% NCP and 36% CP, comparable to the overall distribution of biotype (70% NCP and 30% CP) (Table 5). In cattle that were alive at the time of virus isolation, a higher percentage of NCP isolates (80%) was obtained, as compared to the overall distribution of biotype.

Age of Animal, Biotype, Genotype, and Clinical Signs

Of the 177 BVDV-positive bovine samples, age information at the time of BVDV isolation was available for 122 of the BVDV-positive samples. The majority of animals that were positive for BVDV were less than 1 year of age, accounting for 72% of all BVDV-positive samples. Bovine viral diarrhea virus isolation was most frequent from cattle aged 4–6 months, accounting for 34% of all isolations. For cattle less than 1 year of age, 69% of BVDV-positive samples were obtained from calves that presented dead compared with only 44% of cattle greater than 1 year of age that presented dead.

When information on clinical signs and age of cattle was available, 93 BVDV-positive samples were evaluated (Table 6). In calves 1–3 months of age, respiratory signs (22% of BVDV-positive cases), multiple signs (22% of cases), and others (44% of cases), such as sudden death, lethargy, and neurological sign, were frequent clinical signs. In calves 4–6 months of age, the most frequent clinical signs observed included signs consistent with respiratory disease (41% of cases) and diarrhea (22% of cases). At the age of 7–12 months, diarrhea (39% of cases) and other multiple clinical signs (28% of cases) were frequently observed. In cattle older than 1 year of age, respiratory signs (20% of cases) and diarrhea (20% of cases) were frequent clinical signs.

DISCUSSION

The NCP biotype and BVDV-1 genotype were found in greatest numbers from the BVDV-positive samples examined in this study. These findings are consistent with previous data that indicate BVDV-1 is the more common genotype and NCP is the more common biotype.[2,9,16] In an analysis of 104 Canadian isolates obtained from 1981–1994, 77% of isolates were BVDV-1.[2] A study evaluating 203 BVDV-positive fetal bovine sera indicated that 115 were BVDV-1, 65 were BVDV-2, and 23 contained BVDV-1 and BVDV-2 together.[9] A recent study evaluating the genotypic distribution

for 103 BVDV-positive samples at the Oklahoma diagnostic laboratory from November 1994 to January 1998 indicated that 61% were BVDV-1 and 39% were BVDV-2,[16] and this ratio of BVDV-1 to BVDV-2 is similar to the percentages reported in this study. With respect to biotype, distribution data indicate that NCP isolates are the most common field isolate and account for approximately 65% to 88% of BVDV isolates,[2,13,17] which is similar to our study where NCP isolates accounted for 70% of the BVDV-positive samples. Within each biotype and genotype, there were no differences in the distribution of genotype and biotype, suggesting that distribution of biotype is independent of genotype, and vice versa.

Reproductive failure by BVDV is usually caused by NCP BVDV isolates, and abortion can occur in all periods of gestation.[1] In this study, both genotypes and both biotypes were isolated from aborted fetuses, suggesting that infection of both genotypes and biotypes in pregnant cattle may result in abortion, and that abortion by BVDV is not genotype or biotype specific. Bovine viral diarrhea virus were more frequently isolated from the fetus between the gestational periods of 4–6 months, suggesting that this certain period of gestation may be more susceptible to BVDV infection; however, this finding may be the result of bias, as the fetus between the gestational age of 4–6 months may be more likely to be submitted for a diagnostic work-up compared with early, unobserved embryonic death cases, or late-term abortions and stillbirths.

Diverse clinical signs may be observed in BVDV-infected cattle.[1] Characterizing the clinical description in BVDV-infected cattle is necessary for better understanding of BVDV disease processes. Although clinical signs, such as nasal hemorrhage, oral ulceration, opaque eye, arthritis, neurological signs, lethargy, and sudden death, were observed at low frequency, the major clinical signs of BVDV infection in cattle are respiratory disease, abortion, and diarrhea. In addition, examining the relationship among BVDV genotype, biotype, and clinical signs may provide insight into BVDV pathogenesis. Severe clinical signs associated with BVDV-2 infection have been reported in North America[2]; however, the only clinical finding associated with BVDV-2 in the present study was the relationship of BVDV-2 with oral ulceration. It was the clinical sign reported in the outbreaks of severe clinical disease associated with BVDV-2 infections.[2] Cases in which opaque eyes were observed as the sole clinical sign were associated with only BVDV-1 isolation. The distribution of genotypes for the other clinical signs was proportional to the overall distribution. When comparing biotype with clinical presentation, NCP isolation was associated with oral ulceration, and this finding is similar to previous reports identifying NCP BVDV-2 during the outbreaks of severe BVDV.[2] In addition, CP isolation was more frequent for cases of diarrhea than NCP. Our results suggest that genotype is not clinical sign specific, with possible exceptions (eg, oral ulceration and opaque eyes). Biotype is not clinical sign specific, with possible exceptions (eg, oral ulceration and diarrhea).

The age of the animal at the time of BVDV isolation may be a factor in the outcome of BVDV infection. In this study, a high percentage of BVDV isolation occurred in calves under 1 year of age, suggesting that the majority of clinical disease as a result of BVDV infection occurs in young calves. Of all age groups examined, BVDV isolation was most frequent from calves 4–6 months of age, suggesting that this age may be the most susceptible age group for BVDV infection in terms of overt clinical disease. Frequent virus isolation of this age group may also occur because protective maternal antibody begins to wane, allowing for BVDV infection to occur and calves to become clinical. When examining the clinical signs in calves less than 3 months of age, a high percentage of calves were weak, displaying neurological signs,

arthritis, wasting, and lethargy, which may be a consequence of an in utero BVDV exposure. As calves age, diarrhea and respiratory signs became more frequent. These findings suggest that clinical outcomes may vary depending upon age of cattle.

CONCLUSION

In summary, our results indicate NCP biotype and BVDV-1 genotype are more frequent than CP and BVDV-2. In addition, virulence and clinical presentation may be influenced by factors other than biotype or genotype of the BVDV isolates.

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