Evaluation of horizontal transmission of bovine viral diarrhea virus type 1a from experimentally infected white-tailed deer fawns (*Odocoileus virginianus*) to colostrum-deprived calves

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**Objective**—To assess the transmission of bovine viral diarrhea virus (BVDV) from experimentally infected white-tailed deer fawns to colostrum-deprived calves by use of a BVDV strain isolated from hunter-harvested white-tailed deer.

**Animals**—5 white-tailed deer (*Odocoileus virginianus*) fawns and 6 colostrum-deprived calves.

**Procedures**—Fawns were inoculated intranasally with a noncytopathic BVDV-1a isolate (2 mL containing $10^{6.7}$ TCID$_{50}$/mL), and 2 days after inoculation, animals were commingled until the end of the study. Blood and serum samples were obtained on days –6, 0, 7, 14, and 21 after inoculation for reverse transcriptase PCR assay, virus neutralization, and BVDV-specific antibody ELISA. Nasal, oral, and rectal swab specimens were collected on days 0, 3, 7, 14, 17, and 21 for reverse transcriptase PCR testing. By 21 days after inoculation, all animals were euthanized and necropsied and tissues were collected for histologic evaluation, immunohistochemical analysis, and virus isolation.

**Results**—All fawns became infected and shed the virus for up to 18 days as determined on the basis of reverse transcriptase PCR testing and virus isolation results. Evidence of BVDV infection as a result of cohabitation with acutely infected fawns was detected in 4 of the 6 calves by means of reverse transcriptase PCR testing and virus isolation.

**Conclusions and Clinical Relevance**—On the basis of these findings, BVDV transmission from acutely infected fawns to colostrum-deprived calves appeared possible. (Am J Vet Res 2012;73:257–262)

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**Abbreviations**

BVDV  Bovine viral diarrhea virus

PI  Persistently infected

RT  Reverse transcriptase

VN  Virus neutralization

WTD  White-tailed deer

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capra americana), Canadian bison (Bison bison bison), and moose (Alces alces).8,9,10 The development of clinical signs caused by BVDV infection in wild ruminants is variable and follows the same course as in cattle.11 For instance, in 1 study,12 WTD fawns did not develop any clinical signs as a result of experimental infection with BVDV, however, they actively shed the virus in nasal excretions for up to 7 days after inoculation.

Only 2 studies23,24 have assessed interspecies transmission of BVDV, focusing on transmission by a PI animal. Both studies found that naïve animals became infected with BVDV by being in contact with PI animals. Furthermore, one of the studies24 found that pregnant WTD coming in contact with PI cattle can result in PI fawns. On the other hand, transmission by acutely infected animals is still a matter of controversy. Studies25–27 assessing transmission by acutely infected animals have focused on transmission in cattle or elk (C elaphus) with contradicting results, and there are no studies assessing the possibility of interspecies transmission by acutely infected animals. Additionally, WTD is the most abundant species of wild ruminants in the United States, and BVDV has been isolated from free-ranging WTD.13 The objective of the study reported here was to assess the feasibility of horizontal transmission of BVDV from acutely infected WTD to cattle by commingling them with naïve calves.

Materials and Methods

Animals—Five 2- to 3-week-old female fawns were purchased from a commercial captive deer farm in Indiana. Fawns were fed a commercial doe milk replacer free of anti-BVDV antibodies, feeding intervals varied according to the fawns’ ages. Water and creep feed were offered ad libitum starting at 21 days of age and continuing until the conclusion of the study. Six colostrum-deprived Holstein bull calves were purchased from a large commercial dairy farm in Indiana. At the farm, calves were removed from the dams immediately at birth and placed in separate hutches to prevent colostrum ingestion. Additionally, ear tags were placed and navels were disinfected. Calves were brought to Purdue University Laboratory Animal Housing Facility within 12 hours after birth. For the first 48 hours after birth, calves were fed a human milk replacer every 12 hours, and thereafter, a medicated commercial calf milk replacer was given every 12 hours until the end of the study. In addition to the milk replacer, medicated creep feed was offered ad libitum 7 days after birth until the end of the study.

Virus inoculum—The noncytopathic BVDV-1a strain 544 WTD was used (GenBank accession No. EU597009). This strain was isolated from free-ranging WTD during the Indiana firearm hunting season.14 Virus propagation was performed as described.14

Experimental design—All procedures were approved by the Purdue Animal Care and Use Committee. The number of calves and fawns was mainly restricted by budget limitations. Fawns and calves were housed in the same isolation room under biosafety level 2 at the Purdue University Laboratory Animal Housing Facility. Animals were allowed to acclimate for up to 9 days before the beginning of the study. During this period, blood samples for buffy coat and serum collection were obtained from all animals to test for BVDV types 1 and 2 by means of RT-PCR and VN assays. On arrival, fawns and calves were housed in separate pens. Prior to commingling, all personnel in contact with fawns and calves changed protective clothing, gloves, and boots between handling each species.

As part of the study protocol, starting on arrival day, all calves underwent prophylactic antimicrobial treatment with enrofloxacine (5 mg/kg, SC, q 24 h for 5 days). Additionally, calves received a single dose of 5 g of probiotics PO and a single dose of 3 mL of selenium and vitamin E SC.

Following the acclimation period, all fawns were intranasally inoculated (day 0 of study) with 2 mL of noncytopathic BVDV-1a virus suspension (third passage in cell culture) with a titer of 10^8 TCID50/mL. Two days after inoculation, fawns and calves were commingled until the end of the study. Animals were allocated randomly to 5 groups: 1 group included 1 fawn and 2 calves, and 4 groups included 1 fawn and 1 calf. During this period, animals in the same pen shared feed and water sources.

Clinical examinations performed daily in calves and fawns included measurement of rectal temperature and evaluation of attitude, fecal consistency, and the presence of abnormal respiratory tract signs. A clinical scoring system was used to assign numeric values to daily observations as described.15 The following 4 categories were assessed: lethargy, hemorrhage, respiratory tract signs, and diarrhea. At the time of the clinical evaluations, study personnel were unaware of the diagnostic results.

Blood and serum samples were obtained on days −6, 0 (immediately before inoculation), 7, 14, and 21 for buffy coat samples for RT-PCR and VN assays and BVDV-specific antibody ELISA. Nasal, rectal, and saliva swab specimens were collected on days 0 (immediately before inoculation), 3, 7, 14, 17, and 21 for RT-PCR assay. By 21 days after inoculation, all animals were euthanized by IV administration of a euthanasia solution according to protocol instructions. Following euthanasia, animals were necropsied at the Indiana Animal Disease Diagnostic Laboratory. During postmortem examination, the following samples were collected for histologic evaluation, immunohistochimical analysis, and virus isolation: lymphoid organs (tonsils; retropharyngeal, mandibular, and mesenteric lymph nodes; spleen; and thymus), digestive tract (esophagus, rumen, duodenum, jejunum, and Peyser’s patches in the jejunum, ileum, colon, and rectum), respiratory tract (trachea and lung), heart, skin, and bone marrow. Two samples were collected from each tissue; the first was fixed in neutral-buffered 10% formalin for histologic evaluation, and the second was frozen at −80°C for virus isolation and possible RT-PCR assay.

Virus isolation—Madin-Darby bovine kidney epithelial cells were prepared in 48-well plates grown in 9% (vol/vol) horse serum, 20 mM l-glutamine, and an antimicrobial-antimycotic mixture consisting of penicillin (100 U/mL), streptomycin (10 µg/mL), and
gentamicin (50 µg/mL). Samples (0.25 mL/well) were inoculated in duplicate on cell suspensions and left for 24 hours before culture medium was removed and replaced with new medium. On day 2 after inoculation, cells in duplicate 48-well plates were fixed after cell culture medium was removed by immersing them in cold 80% aqueous acetone for 10 minutes; cells were then evaluated via immunofluorescence microscopy by use of fluorescein isothiocyanate–labeled antibodies specific for BVDV.

BVDV RT-PCR assay and sequence analysis—Quantitative real-time PCR assay was performed on serum, nasal, saliva, and rectal swab specimens as described.\(^{29}\) Viral RNA was extracted from appropriate samples by use of a viral RNA extraction kit as recommended by the manufacturer. Real-time PCR assay was performed on clinical samples as described by targeting the 5'-untranslated region of the viral genome. Real-time PCR assay was performed with a RT-PCR kit in a reaction volume of 25 µL by use of 5 µL of extracted template. Primers were added at a final concentration of 0.4 µM each; the probe was used at a final concentration of 0.2 µM. For quantification, a 1:10 serial dilution of BVDV type 1 preparations of a known virus titer were used to generate a standard curve. The set of standards was included in each run with clinical samples to determine the validity, relative amount, and reproducibility of the assay. The amount of BVDV in each sample was calculated by converting contact time (ie, Ct) value to virus titer by use of the standard curve.

The viral RNA extracted from tissue samples collected at necropsy that were positive by means of virus isolation was then analyzed to verify the degree of homology to the strain used in this study. The set of primers used in the RT-PCR reaction were 103/326. The amplified PCR products were purified by use of a commercial purification kit according to the manufacturer's protocol. These products were sequenced by use of an automated sequencer at the Purdue University genomic core facilities and analyzed, and their homology to the strain used in the present study and other BVDV strains was determined by comparison and reference control viruses. This analysis was determined on the basis of published sequence information and reference control viruses. This analysis was performed by use of computer software.\(^{30}\)

Histologic and immunohistochemical evaluation—Tissues were fixed by immersion in neutral-buffered 10% formalin immediately after collection. Fixed tissues were processed, embedded, and sectioned at 5 µm; each section was stained with H&E.

Immunohistochemical analysis for BVDV in tissues was performed by use of BVDV-specific monoclonal antibodies at the Cornell University Diagnostic Laboratory. In brief, tissue sections (thickness, 5 µm) were deparaffinized, rehydrated, and treated with proteinase K. Each section was incubated with optimally diluted BVDV-specific monoclonal antibodies. Antigen–antibody complexes were stained by use of a biotin–streptavidin–diaminobenzidine system. Sections were counterstained with Gill hematoxylin.

VN—Virus neutralization titers against BVDV were determined as follows: test sera were diluted in a 2-fold series from an initial dilution of 1:4 to a maximum dilution of 1:1,024. Next, 50 µL of inoculum containing 200 TCID\(_{50}\) of BVDV types 1 and 2 was added to each well, and the plates were incubated at 37°C for 2 hours. Fifty microliters of suspension of Madin-Darby bovine kidney epithelial cells at 3 × 10\(^5\) cells/mL was added to each well, and plates were incubated for 3 days at 37°C. Titer was determined via microscopic examination of the monolayer of cells for cytopathic effect. Results were expressed as the reciprocal of the serum 2-fold dilution at which 50% neutralization of virus occurred.

**Results**

Prior to inoculation, 4 fawns and 1 calf became sick from causes other than BVDV infection. One fawn developed oral abscesses that resolved with antimicrobial treatment (20 mg of oxytetracycline/kg, SC, q 48 h); 3 fawns developed diarrhea, which completely resolved in 2 of them. The fawn with intermittent diarrhea throughout the study was euthanized and necropsied on day 16 because of severe physical deterioration. No parasites were detected in fecal samples collected from the sick fawns. However, fecal swab specimens submitted for bacteriologic testing were positive for Escherichia coli. The sick calf developed septicemia as determined on the basis of hyperfibrinogenemia, neutropenia with left shift, and hypopyon observed in the left eye. Results from samples submitted for bacteriologic and parasitological evaluation were negative for this calf. The clinical score was not considered given the fact that some animals were sick before inoculation day and continued to be sick throughout the study period, making it difficult to associate the clinical score with BVDV infection.

All fawns and calves tested negative for anti-BVDV antibodies in VN and ELISA and buffy coat RT-PCR assays prior to the first day of the study. All fawns had evidence of BVDV infection as early as 3 days after inoculation and shed the virus for up to 18 days as determined on the basis of buffy coat RT-PCR assay and nasal, saliva, and rectal swab specimens (Table 1).

Following cohabitation, calves and fawns were commonly seen sharing the same pen area. Four of 6 calves had positive results of buffy coat RT-PCR assay for BVDV. Virus was detected in the buffy coat in one of the calves as early as 5 days and as late as 18 days after inoculation and shed the virus for up to 18 days as determined on the basis of buffy coat RT-PCR assay and nasal, saliva, and rectal swab specimens (Table 1).

Four of the 5 fawns had positive results of VN assays and positive results of the BVDV-specific antibody ELISA. One fawn had positive results of ELISA by day 14 but was seronegative via VN testing. One fawn that was euthanized on day 16 had positive results of ELISA but was seronegative via VN testing. By 21 days after inoculation, the remaining 3 fawns developed VN titers ranging from 1:4 to 1:8 and had positive results of ELISA. Only the calf that had positive results of PCR assay for BVDV after 5 days of cohabitation developed antibodies against BVDV as determined on the basis of positive results of ELISA.

Virus was isolated from the intestines, lungs, or pooled lymph nodes in 3 of the 5 fawns and in 4 calves 21 days after inoculation (19 days after cohabitation;
Table 2—Summary of BVDV RT-PCR assay results in WTD (Odocoileus virginianus) fawns intranasally inoculated with noncytopathic BVDV-1a and commingled with colostrum-deprived Holstein calves.

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<td>–</td>
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<td>C53</td>
<td>+</td>
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<td></td>
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See Table 1 for key.

Table 2). The RNA from these BVDV-positive tissues from the fawns and calves was 99.9% homologous to the strain used in this study as determined on the basis of analysis of the 5'-untranslated region.

On necropsy, no gross lesions were identified in any of the animals. Histologically, all BVDV-infected fawns and calves had marked lymphoid atrophy in the Peyer's patches. No other lesions characteristic of BVDV were observed. Because of budget restrictions, immunohistochemical analysis was performed on an ileum specimen from only one of the BVDV-infected calves. There was positive labeling of BVDV antigen as evidenced by low numbers of scattered positive-staining cells in areas of lymphoid depletion and necrosis and in the lamina propria of villi.

Discussion

Neither fawns nor calves developed any clinical signs related to the infection; this is consistent with results obtained in our laboratory by use of the same BVDV strain. However, 4 fawns were sick within 24 to 48 hours after arrival: 1 fawn developed oral lesions, and 3 fawns developed diarrhea, which resolved in 2 of them. The diarrhea may have been caused by stress from transportation. Escherichia coli was cultured from fecal swab specimens of affected fawns. The calf that was sick 1 week after arrival most likely had septicemia as a result of colostrum deprivation. However, parasitological and bacteriologic results were negative (fecal swab specimens), likely because of antimicrobial use prior to culturing.

All fawns were successfully infected with BVDV following inoculation and were actively shedding the virus as early as 3 days after inoculation and for as long as 18 days in feces, nasal secretions, or oral secretions. The 2 fawns that were sick throughout the study had more severe viremia and shed higher quantities of virus for a longer period of time (data not shown). Concurrent infections in 2 of these fawns may have potentiated the effects of BVDV infection, enhancing the duration of the viremia and amount of virus excreted. In a previous study, BVDV has acted synergistically with bovine rotavirus, worsening the clinical signs in dual-infected calves and resulting in increased BVDV replication. Under natural conditions, concurrent infections may have an effect on BVDV shedding, as in the present study.

Four of the 6 calves were infected with BVDV as a result of direct contact with the fawns as evidenced by the presence of the virus in the buffy coat and in tissues collected at necropsy. Studies assessing BVDV transmission from acutely infected animals to in-contact animals are few. One study found that elk in contact with acutely infected elk actively shedding BVDV resulted in infection of the in-contact animals. However, there was no evidence of shedding of the virus from tissues at necropsy. Only the calf with evidence of infection by 5 days after cohabitating with...
the fawns developed antibody titers against BVDV by 21 days after inoculation as determined on the basis of ELISA results. There is a possibility that the infected calves did not have enough time to develop antibodies, although seroconversion usually occurs within 14 to 30 days after infection.\textsuperscript{33} Microscopic findings agreed with previous studies\textsuperscript{22,27,34,35}, in which the primary histologic lesions observed were lymphoid depletion of Peyer’s patches and thymic atrophy. Compared with previous studies\textsuperscript{34,36,37}, that used virulent BVDV strains, the lack of clinical signs and the paucity of lesions in the present study might be attributable to the low virulence of the strain used.

Colostrum-deprived calves were used in the present study for various reasons. In studies in which animals are challenge inoculated with infectious agents, the susceptibility to infection of those animals should be the same. Because of budget constraints and the fact that approximately 75% of dairy farmers in the United States vaccinate their cattle against BVDV,\textsuperscript{38} it was almost impossible to obtain calves that were free of anti-BVDV antibodies and that were given colostrum.

The authors are aware that this experimental setting did not mimic field conditions. In previous studies, one regional\textsuperscript{39} and another national,\textsuperscript{40} authors reported that approximately 50% of farmers observed either direct wildlife contact or wildlife contact with cattle feed sources, which gives external validity to the present study. Furthermore, the degree of contact between cattle and wildlife varies on the basis of feed and water availability, management systems, and animal density.\textsuperscript{41} Therefore, epidemiologically, the importance of these findings stem from the detection of virus shedding into the environment by both species, which potentially can lead to the infection of a pregnant animal and hence the spread and perpetuation of the virus among both populations. Nevertheless, it should be noted that the latter depends largely on the amount and duration of virus shedding, the infectious dose, duration of virus survival in the environment, population density, and contact frequency between livestock and wildlife.

To the best of our knowledge, this is the first study on transmission of BVDV from acutely infected deer to livestock. In this study, BVDV-infected WTD infected naive calves with BVDV-1a when commingled together for 21 days. On the basis of these findings, wildlife acutely infected with BVDV may be a potential source of infection for susceptible cattle. Field investigations to determine the extent that wild animals contribute to the propagation of this disease would be informative.

References


a. Zoologic Doe Milk Replacer, Pet Ag, Hampshire, Ill.
b. Parent’s Choice, PBM Nutritional, Georgia, Vt.
c. Nurse Chow 100, Purina, St Louis, Mo.
d. Call Starrena, Purina, St Louis, Mo.
f. Probios, Bomanet Vet Plus, Knapp, Wis.
g. BO-SE, Intervet/Shering-Plough Animal Health, Millsboro, Del.
h. IDEXX HerdCheck BVDV antibody ELISA, IDEXX, Westbrook, Me.
j. Sigma Chemical Co, St Louis, Mo.
k. Gibco/BRL Life Science, Grand Island, NY.
l. National Veterinary Services Laboratory, Ames, Iowa.
m. American BioResearch Inc, Seymour, Tenn.
n. YMRD Inc, Pullman, Wash.
o. QIAamp viral RNA extraction kit, Qiagen Inc, Santa Clarita, Calif.
p. Quantitect Probe RT-PCR kit, Qiagen Inc, Santa Clarita, Calif.
q. DNASTAR software, DNA Star Inc, Madison, Wis.


