Bovine viral diarrhea viruses are a group of positive single-stranded RNA viruses that belong to the Pestivirus genus of the Flaviviridae family. Bovine viral diarrhea virus is not host specific, and there is evidence that this virus not only infects cattle but also infects other domesticated ruminant species and wildlife. In fact, its distribution may extend to almost all species included in the order Artiodactyla. Two types of infection are recognized following contact of a naïve animal with BVDV: acute and persistent. Acutely infected animals are believed to be inefficient BVDV transmitters because of the small amounts of virus shed for a short period of time, and these calves eventually clear the virus from their tissues. Conversely, PI animals shed a large amount of virus for long periods, making them the main source of secondary infections. Interestingly, results of a study suggest that BVDV circulates in cattle herds without PI animals. Despite many vaccines available and control programs primarily aimed at the detection and removal of PI animals, BVDV is still endemic in some parts of the world. The fact that BVDV still circulates in cattle populations despite all control measures and evidence that BVDV is present in wildlife warrant further investigation of the transmission at the wildlife-livestock interface.

The presence of BVDV in free-roaming or captive wild ruminants has been documented worldwide either by use of serologic surveys or virus isolation. In North America, the presence of BVDV in the wild ruminant population has been documented in wapiti (Cervus elaphus), mule deer (Odocoileus hemionus), WTD (Odocoileus virginianus), pronghorn antelope (Antilo-
capra americana), Canadian bison (Bison bison bison), and moose (Alces alces).6–10 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 studies23–25 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 experimentally inoculating WTD fawns and 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 antibodiesf, 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 replacerh every 12 hours, and thereafter, a medicated commercial calf milk replaceri was given every 12 hours until the end of the study. In addition to the milk replacer, medicated creep feedj 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.14–16

**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 enrofloxacin (5 mg/kg, SC, q 24 h for 5 days). Additionally, calves received a single dose of 5 g of probiotics POj and a single dose of 3 mL of selenium and vitamin Ek 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 106.7 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.18 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.6 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 solutionn with a titer of 107.7 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.

Animals were necropsied at the Indiana Animal Disease Diagnostic Laboratory. During postmortem examination, the following samples were collected for histologic evaluation, immunohistochemical analysis, and virus isolation: lymphoid organs (tonsils; retropharyngeal, mandibular, and mesenteric lymph nodes; spleen; and thymus), digestive tract (esophagus, rumen, duodenum, jejunum, and Peyers 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 examination, 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 5% (vol/vol) horse serum,19 20mM t-glutamine,4 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. 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 were determined at the monolayer of cells for cytopathic effect. Results from samples submitted for bacteriologic testing were positive for Escherichia coli. The sick calf developed septicemia as determined on the basis of hyperfibrinogemia, 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 cohabitation (Table 1). Only one calf had evidence of the virus in a nasal swab specimen, and another calf had evidence of the virus in 1 oral swab specimen.

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;
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, immuno-histochemical 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.22,23 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,31 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 nasal, oral, and rectal swab specimens. Studies assessing BVDV transmission from acutely infected animals to in-contact animals are few. One study27 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 following infection. Conversely, 2 BVDV transmission studies26,32 between acutely infected calves and naïve calves failed to reveal successful transmission. In both studies, evidence of infection was based on seroconversion and not virus detection.

The presence of antibodies in 4 fawns coincided with clearance of the virus and the inability to isolate 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.\(^{19}\) Microscopic findings agreed with previous studies\(^{22,27,34,35}\) in which the primary histologic lesions observed were lymphoid depletion of Peyer's patches and thymic atrophy. Compared with previous studies\(^{34,36,37}\) 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,\(^{38}\) 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\(^{39}\) and another national,\(^{40}\) 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.\(^{41}\) 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 largely depends 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 naïve 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.

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