

Reproductive tract disease associated with inoculation of pregnant white-tailed deer with bovine viral diarrhea virus

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Objective—To inoculate white-tailed deer (*Odocoileus virginianus*) during the sixth or seventh week of gestation with bovine viral diarrhea virus (BVDV) and observe for signs of reproductive tract disease during a 182-day period.

Animals—10 pregnant white-tailed deer (8 seronegative and 2 seropositive [control deer] for BVDV).

Procedures—Deer were inoculated with 1 of 2 deer-derived BVDV strains (RO3-20663 or RO3-24272). Serum anti-BVDV antibody titers were determined prior to and 21 or 35 days after inoculation. Virus isolation (VI) procedures were performed on tissues from fetuses and does that died and on blood samples collected from live fawns. Ear notch specimens obtained from live fawns were assessed by use of BVDV antigen-capture ELISA (ACE).

Results—Both RO3-20663-inoculated seropositive deer gave birth to apparently normal fawns. Among the RO3-24272-inoculated seronegative deer, 1 died, and 1 aborted and 1 resorbed their fetuses; among the RO3-20663-inoculated seronegative deer, 3 died, 1 aborted its fetus, and 1 gave birth to 2 fawns that were likely persistently infected. On the basis of VI and ACE results, those 2 fawns were positive for BVDV; both had no detectable neutralizing anti-BVDV antibodies in serum.

Conclusions and Clinical Relevance—Reproductive tract disease that developed in pregnant white-tailed deer following BVDV inoculation was similar to that which develops in BVDV-exposed cattle. Methods developed for BVDV detection in cattle (VI, immunohistochemical evaluations, and ACE) can be applied in assessments of white-tailed deer. Fawns from does that had serum anti-BVDV antibodies prior to inoculation were protected against BVDV infection in utero. (*Am J Vet Res* 2008;69:1630–1636)

Bovine viral diarrhea viruses are the causative agent of reproductive, gastrointestinal, and respiratory tract diseases in cattle that result in major losses to the beef and dairy industries. These viruses belong to 2 different species within the pestivirus genus, BVDV1 and BVDV2. The primary consequences of reproductive tract disease are attributable to direct infection of the fetus, and the outcome depends on the stage of gestation in which the fetal infection occurs.¹ Although abortions and weak neonates have been attributed to BVDV infection of cows during late gestation,² infections that

ABBREVIATIONS

ACE	Antigen-capture ELISA
BC	Buffly coat
BSL2	Biosafety level 2
BT	Bovine turbinate
BVDV	Bovine viral diarrhea virus (and also the bovine viral diarrhea virus species within the pestivirus genus)
IHC	Immunohistochemical
MDBK	Madin-Darby bovine kidney
PI	Persistently infected
TCID	Tissue culture infective dose

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develop earlier during gestation generally have greater impact on reproduction. In cattle, fetal infections that develop between 42 and 125 days of gestation result in fetal resorption, mummification, abortion, congenital malformations, or the establishment of PI animals. Persistently infected cattle are considered the main vector for introduction of the virus to naive herds.

Bovine viral diarrhea virus also replicates in white-tailed deer (*Odocoileus virginianus*).^{3–6} Free-ranging white-tailed deer populations are frequently in contact with domestic cattle in the United States; therefore, possible transfer of BVDV between cattle and deer has important implications for proposed BVDV control pro-

grams in cattle. In a previous study,⁵ white-tailed deer fawns that were negative for BVDV and had no detectable serum antibodies against BVDV were inoculated with either a BVDV1 or BVDV2 strain. Both strains used in that study originated from PI white-tailed deer.⁶ Following inoculation, fawns were monitored for febrile response and changes in numbers of circulating lymphocytes and platelets. The progression of clinical signs was similar to that associated with BVDV infections in cattle, including development of fever and depletion of circulating lymphocytes. In another study,⁴ 9 pregnant does were infected with both a BVDV1 (BJ) strain and a BVDV2 (PA131) strain of bovine origin; although no clinical signs of BVDV infection were observed and no abortions detected in any doe, only 1 pregnancy advanced to term, resulting in the delivery of a live PI fawn and a mummified fetus. However, the goal of that study was to determine whether experimental infection of white-tailed deer during gestation with BVDV would result in the birth of PI offspring, rather than to observe all the effects of BVDV on deer reproduction following experimentally induced infection.

The purpose of the study reported here was to inoculate white-tailed deer (*O virginianus*) during the sixth or seventh week of gestation with BVDV and observe for signs of reproductive tract disease during a 182-day period. By keeping the deer in BSL2 containment, observation of individual animals was enhanced, and the risk of exposure to other pathogens during the study was diminished.

Materials and Methods

Virus characterization and propagation—The 2 viruses, RO3-24272 and RO3-20663, used in the study were isolated from 2 different deer carcasses submitted to South Dakota State University for diagnostic testing.⁵ Viruses were propagated in the MDBK epithelial cell line and titrated in BT cells. On the basis of negative results of IHC evaluation⁷ and a PCR assay for BVDV,⁸ both MDBK and BT cells were known to be free of adventitious BVDV. Cells were grown in complete cell culture medium that was composed of minimal essential medium (F15 Eagle medium^a supplemented with 10% fetal bovine serum, L-glutamine [final concentration, 1.4mM], and gentamicin [final concentration, 50 mg/L]). The fetal bovine serum contained no BVDV and no antibodies against BVDV. Viral titers were determined via limiting dilution in BT cells.⁵ Endpoints were based on monoclonal antibodies that bound the BVDV structural protein.⁵

On the basis of phylogenetic analysis of 5' untranslated region sequences (as described previously⁹), strain RO3-24272 was a BVDV1 species and RO3-20663 was a BVDV2 species. Both viruses had a noncytopathic biotype as determined by lack of cytopathic effect in cultured MDBK cells.¹⁰

Animals and experimental procedures—Handling and treatment of deer complied with the Animal Welfare Act as Amended.¹¹ Pregnant does were purchased from a commercial breeder. At time of purchase, the deer were tested for antibodies against BVDV via serum neutralization and for the presence of replicating BVDV

in BC samples via virus isolation. On the basis of the date of contact with a buck, the does were estimated to be at 4 to 5 weeks of gestation at the time of purchase. After purchase, deer were allowed to acclimate to their housing for 10 days prior to viral inoculation. Thus, at inoculation (day 0), the deer were estimated to be at 6 to 7 weeks of gestation. Deer were observed until 182 days after inoculation. Because the duration of gestation in white-tailed deer is typically 193 to 205 days,¹² the end of the observation period for this experiment (182 days after inoculation) extended ≥ 3 weeks longer than the gestation period in this species.

The original design of the study was to inoculate 7 deer with the strain RO3-20663. Clinically mild disease was observed in a preliminary investigation⁵ in which white-tailed fawns were infected with this isolate. However, in the present study, the deaths of 2 of the inoculated does within the first 2 weeks following inoculation raised fears that this virus could be more virulent in adults than originally assumed. For this reason, an additional 3 does were included in the study; these deer were inoculated with the virus RO3-24272 21 days after the first 7 deer were inoculated.

For the duration of the experiment, pairs of deer were housed in pens in a climate-controlled barn that was operated at a BSL2 containment level. Each day, animals were observed a minimum of 2 times, and attitude, cough, loose feces, and signs of abortion were noted. Gestational age of aborted fetuses or age of dead fetuses was estimated on the basis of crown-to-rump length.¹³

Sample collection and assessments—Before inoculation on day 0, a blood sample (25 mL) was collected from each deer to determine pregnancy status, detect serum neutralizing antibodies against BVDV, and assess for BVDV in BC specimens. Another blood sample (15 mL) was collected at either day 21 or 32 after inoculation. The number of blood samples collected was limited in an effort to handle the deer as infrequently as possible. In this species, frequent handling may induce stress to a level that might impact negatively on pregnancy. Each deer was inoculated with 5 mL (1.0×10^6 TCID₅₀/mL) of either RO3-24272 (via the oronasal route) or RO3-20663 (via the oronasal route). This dose was the same as that used in previous studies of BVDV infection in cattle¹⁴ and white-tailed deer.^{4,5} Because BVDV strains cannot be purified, the inoculum consisted of a freeze-thawed lysate of BVDV-infected MDBK cells.¹⁴

Pregnancy was confirmed by use of an ELISA^b that measures the concentration of pregnancy-specific protein B in serum.¹⁵ Serum samples were shipped for analysis at the commercial laboratory^c that produced the test.

Titers of viral neutralizing antibodies in serum were determined by use of a microtiter plate assay; the cytopathic BVDV strains 296c (a BVDV2 species) and TGAC (a BVDV1 species) were included as reference strains. Cytopathic BVDV1 and BVDV2 strains matching the genotypes and subgenotypes of the noncytopathic strains in the inoculums were used in the serum neutralizing antibody tests so that cytopathic effect could be used as the endpoint indicator for serum neutralization data rather than IHC evaluations. Sera

were diluted by serial 2-fold dilutions in complete cell culture medium. A 100- μ L aliquot of diluted serum and a 50- μ L aliquot of virus containing 1,000 TCID were added to each well and incubated for 1 hour at 37°C. At the end of this incubation period, 20,000 BT cells in a 100- μ L aliquot of complete cell culture medium were added to each well. The plate was then incubated for 5 days, after which each well was observed for plaques. Each neutralization assay was done in a replicate of 5—for each dilution of serum, 5 wells that contained the identical dilution of serum were prepared. Titers (endpoint dilutions) were calculated on the basis of the observed cytopathic effect by use of the standard Reed-Münch formula. The endpoint dilutions reflected the highest dilution of serum that inhibited the growth of virus. Titers were recorded as the base 2 logarithm of the reciprocal of the endpoint dilution.

Whereas tissues (including mesenteric lymph nodes, ovaries, spleen, lungs, placenta, whole fetuses, and fetal skin) were collected from any does that died, a complete necropsy was not performed. In fetuses that yielded negative results for BVDV via virus isolation, IHC assessments were performed on samples of skin and spleen. Immunohistochemical assessments were performed on samples of maternal lymph nodes and placenta of does that died after they had developed neutralizing antibodies in serum. To generate fetal homogenates, fetuses were bisected from the head to rump, and half of the body was minced and frozen for virus isolation. Maternal lymph nodes and lungs were minced for virus isolation. Tissues were not cultured for bacteria.

Virus isolation was performed on BC and tissue samples. The BC layer was harvested from whole blood samples collected in evacuated heparinized tubes^d following centrifugation (800 \times g for 20 minutes). The BC samples were frozen at -20°C until processed. To generate the BC inoculum for virus isolation, a 0.5-mL aliquot of the thawed BC sample was mixed with 0.5 mL of complete cell culture medium. For virus isolation in tissue samples, 1 g of minced tissue was frozen at -20°C in 0.5 mL of complete cell culture medium. To generate the tissue inoculum for virus isolation, a frozen sample was mixed with an additional 0.5 mL of complete cell culture medium. One milliliter of BC or tissue inoculum was used to inoculate MDBK cells that had formed a 60% to 70% confluent layer in a 25-cm² flask. After rocking at 37°C for 1 hour, the inoculum was removed from the cells and replaced with 5 mL of cell culture medium (McCoy cell culture medium supplemented with 10% fetal bovine serum). After 5 days, the cell culture (including the medium) was frozen at -80°C. After thawing to 25°C, 1 mL of the resulting lysate was added to a 25-cm² flask containing MDBK cells. After rocking for 1 hour at 37°C, 4 mL of cell culture medium was added. After incubation for 5 days, total RNA was prepared from the culture and tested for BVDV, as described previously.⁹ Any BVDV isolated was compared with the inoculum virus via phylogenetic analysis, as described previously.⁹

Tissue samples for IHC evaluations were fixed in neutral-buffered 5% formalin, processed, embedded in paraffin-based tissue embedding medium, and sectioned as described previously.¹⁵ A blood sample (5 mL)

and an ear notch specimen were collected from each live fawn within 24 hours after birth. Buffy coat samples were harvested and underwent virus isolation, and titers of neutralizing antibodies against BVDV in serum were evaluated as described. Immunohistochemical assessments were performed on cultured uninfected cells to determine whether deer had developed antibodies against the bovine cell proteins in the inoculum. Each ear notch sample collected from fawns was tested for BVDV antigen by use of a commercial ACE^e according to the manufacturer's protocol for evaluation of ear notch specimens from cattle.

Results

Pregnancy status and serum antibody testing before inoculation—All 10 deer were confirmed pregnant at the time of inoculation. Prior to inoculation, titers of serum neutralizing antibodies against BVDV were greater than a value of 1_{log2} in 2 deer (deer 12 and 39). In deer 12, titers of antibodies against a BVDV1 strain and a BVDV2 strain were 9.1_{log2} and 6.9_{log2}, respectively; in deer 39, titers were 10.1_{log2} and 7.3_{log2}, respectively (mean values for the 2 deer, 9.6_{log2} and 7.1_{log2}, respectively). The other 8 deer (deer R1, 3, 4, 22, 23, 16, 28, and O1) were seronegative for BVDV (titers of serum neutralizing antibodies against BVDV were less than a value of 1_{log2}). Antibodies against bovine cultured cell proteins were not detected in serum samples obtained prior to or at days 21 or 32 after inoculation (based on IHC evaluations performed on uninfected tissue culture cells).

Viral inoculations—On day 0, deer R1, 3, 4, 12, 22, 23, and 39 were inoculated with RO3-20663, and deer 16, 28, and O1 were inoculated with RO3-24272. Because of deaths of 2 deer early in the study, the latter 3 deer were subsequently included in the study; these 3 deer were inoculated 21 days after the other 7 deer were inoculated but nevertheless underwent a 182-day postinoculation observation period.

Observations during the first 14 days following inoculation (days 0 to 14)—By day 5, the deer that were initially seronegative for BVDV were less responsive (ie, failed to rise from recumbent positions when handlers walked past their pens), compared with the preceding days' observations. By day 7 after inoculation, these deer had developed signs of depression and an unkempt appearance; their noses were dry and some were drooling.

Two deer (deer 4 and 22) developed signs of depression and died on days 7 and 8, respectively. Samples of the spleen and lymph nodes from these 2 deer yielded positive results for BVDV via virus isolation; virus was also detected in ovary and lung samples from deer 4. Microscopic examination of fixed tissues revealed lymphoid depletion in the spleens of both deer. On the basis of phylogenetic analysis of 5' untranslated region sequences, the viruses isolated from lymphoid tissues matched the inoculum viruses. Although a complete necropsy was not performed on either deer, no oral erosions or ulcerations were evident during a cursory examination of the respiratory and gastrointestinal

tracts. However, both deer had small mesenteric lymph nodes and extensive petechiae in intestinal mucosa. Each doe had 2 fetuses; on the basis of crown-to-rump length, fetal ages ranged from 58 to 62 days (deer 22) and from 59 to 64 days (deer 4). This indicated that the does were at 50 to 56 days of gestation at time of inoculation. Homogenates and skin from all 4 fetuses yielded positive results for BVDV via virus isolation. Immunohistochemical evaluations of tissues from these deer and fetuses were not performed. The cause of death of deer 4 and 22 could not be unequivocally determined from the data collected.

Observations at days 15 through 119—Analyses of serum samples obtained on days 21 or 32 revealed that all surviving inoculated deer (deer R1, 3, 12, 23, 39, 16, 28, and O1) had mounted an immune response to BVDV; compared with findings prior to inoculation, titers of serum neutralizing antibodies against BVDV had increased in the second sample (Table 1). On day 21, it was confirmed that deer R1, 3, 12, 23, and 39 were pregnant; pregnancy status was not assessed in deer 16, 28, or O1 at any point after inoculation. Deer O1 had bloody vaginal discharge on day 39; however, no testable material was recovered, and at the end of the study (day 182 after inoculation), deer O1 was not pregnant.

On day 53, a third doe (deer R1) died apparently as a result of septicemia. This deer was seronegative for BVDV prior to inoculation but had a titer of serum neutralizing antibodies against the virus that was $> 5_{\log 2}$ at day 21. Deer R1 had 2 fetuses, which were too decomposed to allow estimation of gestational age. This deer

had been treated with florfenicol^f and flunixin meglumine^f in the weeks prior to death because of swollen limb joints in the forelimbs and draining abscesses of the right forelimb and neck. Virus isolation and IHC evaluations of tissue samples collected from deer R1 were negative for BVDV; fetal tissues could not be tested because of decomposition. Although the data do not allow one to draw any conclusions as to the cause of death of the fetuses, the advanced decomposition indicated that the fetuses died prior to the death of the doe. Thus, the 1 conclusion that can be supported is that the deaths of the fetuses were not a direct result of the death of the doe.

On day 79, a fourth doe died (deer 16). It had been eating apparently normally, and no changes in behavior were evident prior to death. This deer was seronegative for BVDV prior to inoculation but had a titer of serum neutralizing antibodies against the virus that was $> 5_{\log 2}$ at day 21. No gross lesions in the doe were detected when fetal tissues were collected for virus isolation and IHC evaluations. The development of a high titer of serum neutralizing antibodies suggested that this doe had mounted an immune response that was capable of clearing the virus. For this reason, tissues were not collected from the doe for virus isolation and IHC evaluations. Virus was not isolated from fetal homogenates or skin samples; results of IHC evaluations of fetal specimens were negative. The twin fetuses appeared to have died at different stages of gestation, and based on the condition of the fetuses, their deaths preceded that of the doe. This conclusion was supported by the estimated fetal ages (72 to 80 days and 88 to 93 days), compared with the estimated duration of gestation (121 to 128 days on the basis of exposure to the buck) at the time of the doe's death. Thus, the fetuses appeared to have died 5 to 7 weeks prior to the death of the doe.

Observations at days 120 through 182—At day 139, deer 23 expelled 2 mummified fetuses. The estimated fetal ages were 75 to 83 days. However, it should be noted that the method of gestational age estimation on the basis of crown-to-rump length was derived from data collected from fresh fetuses; to our knowledge, there is no similar method of gestational age estimation for mummified fetuses. Thus, the method derived from data collected from fresh fetuses was used to provide an age estimate of the mummified fetuses with this known limitation. The mummified state of the fetuses suggested that the fetuses died some time before they were expelled from the doe. Features of fetal development in these 2 fetuses were consistent with those expected in fetuses at days 75 and 93 of gestation.^{12,13}

On the basis of the duration of gestation in this species (193 to 205 days),¹² the estimated stage of gestation at inoculation (6 to 7 weeks), and the inclusion of a 2-week interval to account for early or late births, we predicted that parturition would occur approximately 130 to 160 days after inoculation. The first fawn was born 128 days after inoculation; prior to inoculation, the doe (deer 39) had titers of serum antibodies against both BVDV species that were $\geq 9_{\log 2}$. Deer 12 gave birth to twins at 147 days after inoculation; prior to inoculation, this deer also had titers of serum antibodies against both BVDV species that were $\geq 9_{\log 2}$.

Table 1—Titers (recorded as the base 2 logarithm of the reciprocal of the end point dilution) of serum neutralizing antibodies against BVDV prior to inoculation with BVDV (day 0) and at day 21 or 32 after inoculation in pregnant white-tailed deer that lived to the end of the postinoculation observation period (182 days) and outcome of those pregnancies. Does were inoculated with 1 of 2 BVDV viruses (RO3-24272 [BVDV1 species; deer 28 and O1] via the nasal route or RO3-20663 [BVDV2 species; deer 3, 12, 23, and 39] via the oronasal route); 5 mL of 1.0 × TCID₅₀ of virus/mL was administered as each inoculum.

Deer	Titer (log ₂ value)				Outcome of pregnancy
	Prior to inoculation		At day 21 or 32 after inoculation		
	BVDV1	BVDV2	BVDV1	BVDV2	
12	9.1	6.9	14.9	12.5	Apparently normal twin fawn born at day 147
39	10.1	7.3	13.9	12.9	Apparently normal fawn born at day 128
O1	0	0.9	16.1	10.1	Bloody vaginal discharge evident at day 39; no fetal material recovered for testing
23	0	0	2.9	5.5	Abortion detected at day 139; fetuses were mummified (gestational age estimated at 75–83 days)
28	0	0	10.9	2.5	Pregnant at beginning of study but not pregnant at day 182
3	0	0	0	7.1	2 Pl live fawns born at day 163

These 3 fawns, which were normal in appearance and apparently healthy, yielded negative results for BVDV via virus isolation; BVDV antigen in ear notch specimens and anti-BVDV antibodies in serum were not detected. At 163 days, deer 3 gave birth to 2 undersized fawns. These 2 fawns yielded positive results for BVDV in BC samples via virus isolation, BVDV antigen was detected via ACE, and there was no evidence of neutralizing anti-BVDV antibodies in serum. These fawns were smaller than the fawns borne by deer 39 and 12 that were seropositive for BVDV at the time of inoculation. During the night following their birth, both of deer 3's fawns died. Evidence of trauma (contusions and skin wounds) suggested that an attack by the doe may have contributed to their deaths. On the basis of findings of phylogenetic analysis, the viruses isolated from these 2 fawns were identical and matched the inoculum virus (RO3-20663) given to the doe. Skin samples collected from each fawn after death were positive for BVDV antigen as determined via IHC evaluation.

As stated previously, deer O1 had bloody vaginal discharge on day 39 but had not given birth by day 182. Examination of the reproductive tract via palpation at day 182 revealed that this doe was not pregnant. Similarly, another doe (deer 28) that was confirmed pregnant at time of inoculation did not deliver a live fawn or abort a fetus and was not pregnant (determined via palpation) at 182 days after inoculation.

Overall, only 3 of the 10 deer gave birth to live fawns. Two of the does (deer 12 and 39) that delivered live fawns had serum antibodies against BVDV at the time of inoculation. Fawns born to these 2 deer yielded negative results for BVDV in BC samples via virus isolation and for BVDV antigen in ear notch specimens. Deer 3 gave birth to BVDV-antigen-positive fawns that also yielded positive results for BVDV via virus isolation. In contrast to deer 12 and 39, deer 3 was seronegative for BVDV at the time of inoculation. Among the does that did not give birth to live fawns, fetal deaths appeared to occur at 58 to 93 days of gestation (corresponding to days 7 through 49 after inoculation of the does with BVDV). The fetus of deer 28 was apparently resorbed because the doe was confirmed pregnant at time of inoculation, developed no sign of abortion, did not give birth to a live fawn, and was not pregnant at the end of the observation period.

Discussion

In the present study, a high degree of reproductive failure was evident among the seronegative pregnant white-tailed deer exposed to BVDV. This observation is consistent with that of a previous study⁴ in which 9 pregnant deer were inoculated with BVDV. In that study, the reproductive failure rate was > 80%. In contrast, the 2 does in the present study that were seropositive for BVDV (titers of serum neutralizing antibodies $\geq 9_{\log_2}$) both gave birth to apparently normal, live fawns. This is consistent with a study¹⁶ in cattle in which a correlation between the presence of serum neutralizing antibodies against BVDV and protection against fetal infection with the virus was reported.

The death loss among the deer was unexpected in our study. In a previous investigation,⁵ fawns were ex-

posed to the viruses used in the present study and developed only mild clinical signs of disease. The stress of pregnancy may have contributed to more severe clinical outcome among the adult females. It is possible that fetal death impacted maternal health, resulting in the deaths of certain does. The cause of death in the 2 deer that died on days 7 and 8 could reasonably be attributed to acute BVDV infection because the deer were housed in a BSL2 containment area, there was no evidence of other pathogens, and BVDV was isolated from various tissue samples. The timing of death with respect to BVDV inoculation correlates with the period of viremia associated with BVDV infection in cattle.

Deaths of deer R1 and 16 (which died on days 53 and 79, respectively) cannot be directly attributed to BVDV on the basis of the results of virus isolation in maternal and fetal tissues. The fetuses of deer R1 and 16 apparently died prior to the death of either doe itself. It is possible that BVDV infection contributed to the deaths of the fetuses, which in turn contributed to the deaths of the deer. However, fetal tissues for testing were not recovered from one of the deer, and the fetal tissues from the other were negative for BVDV (as determined via virus isolation); thus, BVDV infection of these fetuses cannot be confirmed. Similarly, BVDV cannot be unequivocally linked to the deaths of the mummified fetuses of deer 23 or the apparent abortion of the fetus of deer O1. However, the postinoculation development of neutralizing anti-BVDV antibodies in all 4 of these initially seronegative deer (deer R1, 16, 23, and O1) suggests that virus replication took place.

The outcome of BVDV-associated fetal infection in cattle is dependent on the stage of gestation; in 1 study,¹ calves became PI following infection of fetuses within the first 125 days of gestation. Although 125 days is often quoted as the gestational age cutoff for development of persistent infection, another study¹⁷ revealed that most PI calves were more likely infected within the first 100 days of gestation and that infection of fetuses between 100 and 125 days of gestation rarely resulted in PI calves. After 125 days of gestation, although BVDV infection can be cleared by a fetus, congenital anomalies¹ may result that could lead to the death of the fetus. It is generally difficult to isolate BVDV or detect viral antigen in calves that are infected in utero after 125 days of gestation.¹ To the authors' knowledge, the range of gestational age in which deer fetuses may become PI is undetermined. The duration of gestation in deer is approximately 33% less than that in cattle. If one makes the assumption that the period in which deer fetuses may become PI is shortened proportionally, then the upper limit of gestational age would be approximately 83 days; furthermore, fetuses at < 67 days of gestation would be more likely to become PI. The failure to isolate BVDV from the fetuses of deer 16 (specimens collected after the death of the deer) and the mummified fetuses aborted by deer 23 could indicate that BVDV did not infect the fetuses; that BVDV infected the fetuses, which were subsequently able to eliminate virus; or that BVDV infected the fetuses, but the virus did not survive in tissues during the 20- to 30-day period between the death of the fetuses and the

(deer 16) or during the 50- to 60-day period between the death of the fetuses and their expulsion from the doe (deer 23).

The development of neutralizing antibodies against BVDV indicated that the virus replicated in both deer 16 and 23. It is possible that the virus replicated in these does but did not cross the placenta. Given the nature of the virus and the lack of antibodies against BVDV in these 2 deer at the time of inoculation, this does not seem highly likely; results of experimental infections in cattle have indicated that BVDV crosses the placenta with 100% efficiency in naive animals.^{18,19}

The apparent abortion in deer O1 could not be directly traced to BVDV because fetal material for testing was not recovered. Again, viral replication appeared to occur in this doe on the basis of the development of neutralizing anti-BVD antibodies in serum.

Fetal death, abortion, and fetal mummification are consistent with features of BVDV-induced reproductive tract disease in cattle. In the present study, abortions did not occur in the 2 deer that were seropositive for BVDV prior to inoculation, and housing of the deer in a climate-controlled BSL2 containment area lessened the likelihood that other pathogens or environmental stress contributed to reproductive failure. The estimated age of the mummified fetuses was 75 to 83 days of gestation, which indicated that they died 3 to 4 weeks following the inoculation of deer 23 with BVDV. This finding is similar to that of a previous study¹⁹ of BVDV infection of pregnant cattle, in which it was observed that 1 BVDV-inoculated cow retained a mummified fetus until day 300 of its pregnancy; the fetus had died at approximately 100 days of gestation.

Deer 3 was seronegative for BVDV prior to inoculation and gave birth to the only live fawns in which BVDV antigen was identified in skin specimens via IHC evaluation and ACE, BVDV was isolated from BC samples, and serum neutralizing anti-BVDV antibodies were not detected. These findings are consistent with these 2 fawns being PI with BVDV. Typically, confirmation that an animal is PI requires collection and testing of samples again after a 3-week interval, the results of which should be positive for virus or viral antigen. Unfortunately, these fawns did not survive long enough to allow repeat testing. However, the IHC evaluations and ACE performed on ear notch samples rarely detect acute infections.²⁰⁻²² Additionally, deer 3 had been held in BSL2 confinement for more than 5 months prior to the fawns' birth and had developed neutralizing antibodies against BVDV after inoculation with the virus. These 2 facts indicate that a new exposure to BVDV and the spread of the virus from the doe to the fawns in the 2 weeks prior to their birth were unlikely to occur.

Results of the present study of pregnant white-tailed deer inoculated with BVDV during the sixth or seventh week of gestation indicated that infection of fetuses with BVDV can develop within 7 to 8 days after inoculation. Inoculation with BVDV resulted in abortion and mummification of fetuses and the birth of apparently PI fawns. However, the fawns of does that had serum neutralizing antibodies against BVDV at the time of inoculation were protected from infection. These observations are consistent with the clinical features of

BVDV-associated reproductive tract disease in pregnant cattle that are exposed to the virus before 125 days of gestation. Further research needs to be done to determine whether signs of BVDV-associated reproductive tract disease in white-tailed deer and in cattle are similar at later stages of gestation and to determine, more exactly, the period during gestation in which white-tailed deer fetuses are susceptible to development of persistent BVDV infection.

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 - b. BioPRYN, Biotracking LLC, Moscow, Idaho.
 - c. Biotracking LLC, Moscow, Idaho.
 - d. Vacutainer sodium heparin tubes, Becton-Dickinson, Franklin Lakes, NJ.
 - e. Herdchek, Idexx Laboratories, Westbrook, Me.
 - f. Schering-Plough Animal Health, Summit, NJ.
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