Prevention of persistent infection in calves by vaccination of dams with noncytopathic type-1 modified-live bovine viral diarrhea virus prior to breeding

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Objective—To determine the ability of a modified-live virus (MLV) bovine viral diarrhea virus (BVDV) type 1 (BVDV1) vaccine administered to heifers prior to breeding to stimulate protective immunity that would block transmission of virulent heterologous BVDV during gestation, thus preventing persistent infection of a fetus.

Animals—40 crossbred Angus heifers that were 15 to 18 months old and seronegative for BVDV and 36 calves born to those heifers.

Procedure—Heifers were randomly assigned to control (n = 13) or vaccinated (27) groups. The control group was administered a multivalent vaccine wherein the BVDV component had been omitted. The vaccinated heifers were administered a single dose of vaccine (IM or SC) containing MLV BVDV1 (WRL strain). All vaccinated and control heifers were maintained in pastures and exposed to BVDV-negative bulls 21 days later. Thirty-five heifers were confirmed pregnant and were challenge exposed at 55 to 100 days of gestation by IV administration of virulent BVDV1 (7443 strain).

Results—All control heifers were viremic following challenge exposure, and calves born to control heifers were persistently infected with BVDV. Viremia was not detected in the vaccinated heifers, and 92% of calves born to vaccinated heifers were not persistently infected with BVDV.

Conclusions and Clinical Relevance—These results document that vaccination with BVDV1 strain WRL protects fetuses from infection with heterologous virulent BVDV1. (*Am J Vet Res* 2003;64:530–537)

Bovine viral diarrhea virus (BVDV) is an enveloped, positive-strand RNA virus that is a member of the *Pestivirus* genus. Bovine viral diarrhea virus has a worldwide distribution and is responsible for substantial economic losses in cattle. Infection with BVDV in susceptible cattle generally causes mild fever, leukopenia, and respiratory or gastrointestinal tract disease, followed by an increase in serum neutralizing antibodies developed against the virus.[1-3] Because of the potential immunosuppressive effects of BVDV, the virus may also enhance the infectivity of other viral and bacterial pathogens. Strains of BVDV are antigenically variable. Virus strains have been classified into 2 genotypes (**BVDV type 1 [BVDV1]** and **BVDV type 2 [BVDV2]**) on the basis of differences in the genomic sequence.[4] The BVDV2 strains have been associated with acute hemorrhagic disease; however, virulence is variable in both genotypes.[5-9] Strains of BVDV also vary in their ability to cause cytopathic effects in cell culture. Both cytopathic and noncytopathic strains can infect cattle, whereas only noncytopathic strains have the ability to persistently infect cattle.[10,11]

Acute BVDV infection of pregnant cattle generally causes subclinical disease, and the virus readily spreads via the placenta. The outcome of fetal infection is dependent on virulence of the strain of BVDV and age of the fetus at time of infection.[1-3] Early in gestation, BVDV infection can cause fetal resorption or abortion. At approximately 150 to 200 days of gestation, fetuses become capable of mounting immune responses to BVDV antigens. Fetuses infected in utero after this time can seroconvert to BVDV and clear the infection. Transmission of BVDV to the fetus at approximately 60 to 125 days of gestation can lead to establishment of persistent infection of the calf, a condition in which it is specifically immunotolerant to the infecting strain of BVDV. Such persistently infected calves may appear clinically normal at birth, they may be unthrifty, or they could have congenital defects such as cerebellar hypoplasia.[12]

Persistently infected cattle do not produce neutralizing antibodies against the specific infecting strain of BVDV, are viremic, continuously shed BVDV into their environment,[12,13] and transmit the virus to other cattle.[1] The prevalence of persistently infected cattle has been estimated at approximately 1%.[10,14,15] These persistently infected cattle represent the primary reservoir of BVDV in the environment.[16]

Persistently infected cattle may also develop fatal mucosal disease.[1-3] Mucosal disease can be induced when the persistent noncytopathic strain undergoes genomic rearrangement attributable to addition of a protease cleavage site by insertion of cellular sequences

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or duplication of viral sequences. This genomic rearrangement leads to the cytopathic phenotype and a noncytopathic-cytopathic virus pair in the calf.[17,18] Mucosal disease generally results in severe clinical signs of disease with a high mortality rate.

Currently, licensed commercial vaccines in the United States are required by the USDA to demonstrate safety and efficacy against experimental challenge exposure to BVDV. There is not a requirement for efficacy in protecting fetuses against persistent infection. However, a vaccine effective in protecting fetuses against persistent BVDV infection would result in reduction of BVDV shedding in the environment, thereby reducing exposure of susceptible cattle to the virus by reducing the number of persistently infected animals in the population. Thus, the objective of the study reported here was to determine the ability of a **modified-live virus (MLV)** BVDV1 vaccine administered to heifers prior to breeding to stimulate protective immunity that blocks transmission of virulent BVDV during gestation, thus preventing persistent infection in the fetus.

Materials and Methods

Animals—Forty crossbred Angus heifers that were 15 to 18 months old and 2 beef-type crossbred bulls were used in the study. Reproductive examinations by per rectal palpation were performed on all heifers before initiation of the study. For 21 days after vaccination, control heifers were penned so that there was no physical contact with vaccinated heifers. During this period, all human contact was arranged so that the handlers dealt with the control heifers before proceeding to the vaccinated heifers. Subsequently, vaccinated and control heifers were commingled for the remainder of the study.

Vaccination—Heifers were assigned to blocks on the basis of body weight, and each block was randomly assigned to a treatment group (13 control heifers and 27 vaccinated heifers). Vaccine containing BVDV1 (WRL strain) and MLV infectious bovine rhinotracheitis virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus was administered. The BVDV titer in response to vaccination was determined by 5 replicate titrations at the time of vaccination by use of a microtiter direct **immunofluorescence assay (IFA)** on a **bovine kidney (BK)** cell line. For BVDV titrations, the multivalent vaccine was incubated for 1 hour at room temperature (approx 22°C) with a mixture of monospecific antisera prepared against infectious bovine rhinotracheitis virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus to block these fractions in the titration assay. Monolayers of BK cells that had been incubated for 24 hours in 96-well plates were inoculated with dilutions of blocked vaccine virus. Four days after inoculation, plates were fixed and stained by use of a BVDV-specific direct IFA conjugate.[a] Wells were scored as positive or negative for BVDV, and titer (**50% fluorescent antibody infectious dose [$FAID_{50}$]**) was calculated by a method reported elsewhere.[19] Titer of BVDV in the vaccine was 3.0 $\log_{10}FAID_{50}$/dose.

Vaccine was administered on day 0 as a 2-mL dose to 14 heifers by IM injection and 13 heifers by SC injection. Vaccine was injected approximately 5 to 10 cm cranial to the right scapula. Thirteen control heifers received a sham vaccination by injection of a solution that was identical to the test vaccine but without the BVDV fraction; control heifers were injected IM in the same anatomic location as vaccinated heifers. The vaccine was stored on wet ice until BVDV titration, which was performed immediately after the final vaccination.

Breeding—Heifers were maintained in pastures; bulls were added to the population of those pastures on days 21 to 66. Per rectal palpation of the heifers was performed on day 119 or 120 to determine pregnancy. Thirty-six heifers were confirmed pregnant, and 4 heifers were removed from the study prior to challenge exposure, because they were not pregnant. Thus, overall conception rate was 90%.

Experimental challenge—The BVDV1 strains WRL and Singer were propagated on **Madin-Darby bovine kidney (MDBK)** cells. Virus used for challenge exposure (BVDV1 strain 7443) was obtained from the National Animal Disease Center, Ames, Iowa. Strains WRL and 7443 are noncytopathic, and the Singer strain is cytopathic. Virus was propagated on bovine turbinate cells. Cells were propagated in **eagle modified Earle's medium (EMEM)**[b] supplemented with 10% adult bovine serum.[c] Virus propagation was performed in the same medium but with serum concentration reduced to 0.7 to 2.0%. All cell cultures and sera were determined to be free of *Mycoplasma* spp, BVDV, and BVDV antibody.

Virus was administered to heifers on day 121 (day 55 to 100 of gestation) for challenge exposure. A 2-mL dose of virus (6.5 $\log_{10}FAID_{50}$) was administered IV. One heifer (vaccinated IM) was removed from the study on day 163 because of an injury resulting from handling at the time of challenge exposure. Therefore, 35 heifers completed the study (11 control heifers, 11 heifers vaccinated IM, and 13 heifers vaccinated SC).

Clinical evaluation—Heifers were observed and rectal temperatures obtained once daily starting 2 days prior to challenge exposure and ending 14 days after challenge inoculation. Heifers were observed for signs of respiratory tract disease, nasal discharge, ocular discharge, attitude, and gastrointestinal tract disease. Calves were weighed and underwent a complete physical examination as soon after birth as possible.

Hematologic examination—Blood samples were collected from heifers daily for 3 days prior to challenge exposure and every other day during the 14-day observation period after challenge exposure. Samples were analyzed for total WBC count. A baseline WBC count was determined for each heifer by determining the mean value for the daily counts of the 3 days immediately prior to challenge inoculation. Mean WBC counts for each experimental group were calculated.

Serum neutralization assay—Blood was collected, and serum was harvested and heat-inactivated (45°C for 1 hour) for determination of the BVDV titer. A microtiter assay method was used with BK cell monolayers, similar to that described for the titration assay. Four wells were inoculated per serum dilution. Singer strain of BVDV was used in the **serum neutralization (SN)** assay. Because the Singer strain is cytopathic, visual cytopathic effect was used to calculate the SN titer. Results were expressed as the 50% endpoint dilution that neutralized 100 to 300 $TCID_{50}$ of BVDV1, as calculated by a method described elsewhere.[19]

Virus isolation—Blood was collected into serum separator tubes[d] and allowed to clot for 2 hours at approximately 22°C. Serum was removed and stored at –70°C. Nasal-swab specimens were collected by use of polyester-tipped swabs[e] and placed into 3 mL of swab medium; swab medium consisted of EMEM containing neomycin sulfate[f] (25 µg/mL), gentamicin sulfate[g] (50 µg/mL), penicillin[h] (25 U/mL), streptomycin[i] (25 U/mL), and bacitracin[j] (0.25 U/mL). Samples were stored at –70°C. Blood was collected into heparinized tubes[k] and stored on wet ice until processed for evaluation of the buffy coat. Buffy coats were harvested by centrifugation of blood samples (700 to 800 × *g* for 10 minutes at 4°C).

Buffy coat cells were collected after aspiration of the plasma layer and dilution with an equal volume of EMEM. The cell suspension was layered onto 4 mL of sodium diatizoate-polysucrose[l] and centrifuged at 700 to 800 × *g* for 40 minutes at 4°C. Cells at the interface were collected and washed 3 times with serum-free EMEM and then washed a final time in EMEM containing 10% adult bovine serum. The washed cell suspension was adjusted to a concentration of 2×10^6 cells/mL by adding EMEM; the final suspension was stored at –70°C until immediately prior to testing. Samples were inoculated onto 25-cm^2 monolayers of MDBK cells; cell culture fluids were harvested 4 to 5 days after inoculation.

For selected calves, samples of liver, mesenteric and prescapular lymph nodes, spleen, thymus, and small intestine near the ileocecocolic junction were aseptically collected, placed in sterile plastic bags, and stored at –70°C until testing. Tissues were thawed rapidly, and 1 to 2 cm^3 of tissue was placed in a mortar with sterile sand[m] and approximately 3 mL of swab medium. Tissue was ground with a pestle, and the fluid was transferred to a tube and centrifuged at 250 × *g* for 5 minutes at 4°C. Supernatant was collected and stored on wet ice until testing. Samples were inoculated onto 25-cm^2 monolayers of MDBK cells; cell culture fluids were harvested 4 to 5 days after inoculation.

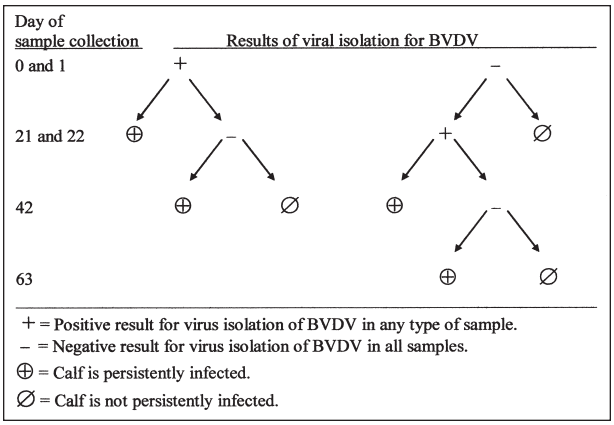

Figure 1—Schematic diagram of the method used to classify calves as persistently infected with bovine viral diarrhea virus (BVDV). Serum samples, nasal-swab specimens, and buffy coat cells used to test for BVDV were obtained from calves prior to ingestion of colostrum on the day of birth (day 0), on day 1, and at approximately 3 week intervals until a calf was classified as persistently infected or not persistently infected. Samples were obtained on 2 consecutive days at 3 weeks of age. Calves were classified as persistently infected when they had 2 positive results on samples obtained at least 3 weeks apart (but not necessarily on samples obtained at consecutive collection periods).

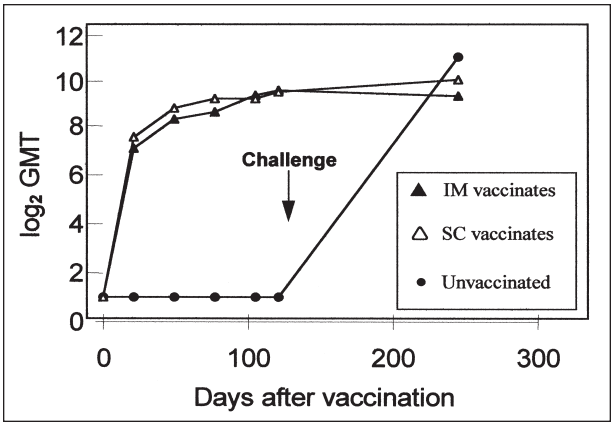

Figure 2—Geometric mean serum-neutralizing antibody titer (GMT) to BVDV type 1 (BVDV1) in heifers that were not vaccinated or were vaccinated IM or SC with a modified-live virus vaccine of BVDV1 (strain WRL) and subsequently challenge exposed with BVDV1 (strain 7443).

Virus isolation of each processed sample was performed by inoculation of undiluted sample and a diluted sample (dilution, 1:10) directly onto 96-well plates (4 wells/sample or dilution) that were seeded with BK cells (1×10^5 cells/mL; 0.1 mL/well). Plates were incubated for 4 to 5 days and then fixed. Then, a direct IFA assay was performed by use of a BVDV-specific direct IFA conjugate,[a] as described previously for the titration assay. Wells positive for BVDV were visually identified and recorded. A sample was considered to have positive results when 1 or more wells had IFA staining.

Diagnosis of persistent infections—To diagnose a persistent infection in a calf, nasal-swab specimens, serum, and blood samples were obtained from each calf prior to the first colostral feeding on the day of birth. Nasal-swab specimens, serum, and blood samples were again obtained from each calf at day 1 and at approximately 3-week intervals until the calf was classified as persistently infected or not persistently infected. Samples were obtained from all calves on days 0 and 1, and samples were obtained on 2 consecutive days at 3 weeks of age. Subsequent days of sample collection were adjusted slightly so that several calves could be grouped for ease of collection, processing, and shipping of samples. Calves that had 2 positive results for viral isolation of BVDV on samples obtained at an interval of at least 3 weeks (but not necessarily on samples obtained at consecutive collection periods) were considered persistently infected (**Fig 1**). Calves that had negative results for viral isolation of BVDV on 2 consecutive samples obtained 3 weeks apart were considered not persistently infected. All calves were euthanized after their classification was established. One calf died before it was 3 weeks old; additional virus isolations were performed on tissues collected from that calf at the time of necropsy.

Statistical analysis—Differences in amount of protection among calves born to vaccinated or control heifers were evaluated by use of the 2-tailed Fisher exact test.[20,n] Analysis of WBC counts was performed by use of an ANCOVA, using the mean WBC count for days 119 to 121 (before challenge exposure) as the covariate. Pairwise analysis of treatment means was conducted by use of least-squares means. Values of $P \leq 0.05$ were considered significant.

Results

Serologic evaluation—All heifers were seronegative to BVDV (SN antibody titer, < 1:2) on the day of vaccination (**Fig 2**). All control heifers remained seronegative until after challenge exposure. All vaccinated heifers seroconverted (SN titer, 1:64 to 1:724) by day 21. Antibody titers continued to increase throughout the postvaccination period. On the day of challenge exposure, SN titers for vaccinated heifers ranged from 1:362 to 1:1,024. All control heifers seroconverted after challenge exposure (SN titer, 1:512 to > 1:2,048).

Clinical observations—Mild serous nasal discharge was observed in some heifers of both groups prior to the day of challenge exposure and throughout the postchallenge observation period (data not shown). Additionally, 1 control heifer had mucopurulent nasal discharge on days 4 through 9 after challenge exposure, and 3 control heifers had mild diarrhea in the postchallenge period. In the group vaccinated IM, 4 heifers had mild diarrhea for at least 1 day in the postchallenge

period. One heifer also was observed coughing for a single day; this heifer was later removed from the study because of injuries sustained during handling. In the group vaccinated SC, 3 heifers had mild diarrhea, and 1 heifer had mucopurulent nasal discharge in the postchallenge period. In addition, 1 heifer was slightly lethargic on 1 day, and another had ocular discharge on the last 2 days of the observation period.

Two control heifers had rectal temperatures of 39.7 and 40.3°C, respectively, on day 7 after challenge exposure. None of the heifers vaccinated SC had an increase in rectal temperature (> 39.4°C) during the observation period. One of the heifers vaccinated IM had an increase in rectal temperature (> 39.4°C) between days 7 and 14 after challenge exposure that was associated with an injury sustained during handling; this heifer was subsequently removed from the study.

Hematologic examination—The WBC counts in control heifers decreased 2 days after challenge exposure (**Fig 3**). Mean WBC count for the control heifers on the day after challenge exposure was 4,800 cells/µL (52% of the mean baseline count). Mean WBC counts for the control heifers remained less than the baseline count throughout the sample collection period. Seven of 11 control heifers were leukopenic on at least 1 day

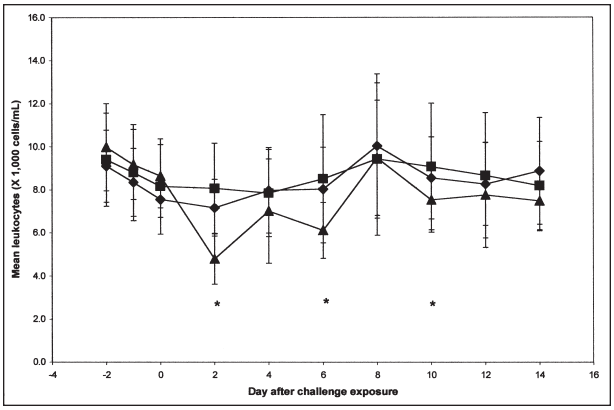

Figure 3—Mean ± SEM WBC counts after challenge exposure with BVDV1 (strain 7443) in control heifers (triangle) and heifers vaccinated IM (diamond) or SC (square) with modified-live BVDV vaccine. *Value differs significantly ($P < 0.05$) between control and vaccinated heifers.

Table 2—Results* of virus isolations for BVDV from 11 calves born to control heifers

Calf	Day of age†	Type of sample: Serum	Nasal-swab specimen	Buffy coat cells
476B	0	+	+	+
	1	+	–	+
	20	–	+	+
	21	–	–	+
478B	0	+	+	+
	1	–	+	+
	20	+	+	+
	21	+	+	+
484B	0	+	+	+
	1	–	–	+
	21	–	+	+
	22	–	+	–
485B	0	+	+	–
	1	+	+	+
	22	+	+	+
	23	+	+	+
496B	0	+	+	+
	1	+	+	+
	24	–	+	+
	25	–	+	+
497B	0	+	+	+
	1	+	–	+
	22	+	+	+
	23	+	+	+
499B	0	+	+	+
	1	+	–	+
	21	+	+	+
	22	+	+	+
503B	0	+	+	+
	1	+	+	+
	21	+	+	+
	22	+	+	+
507B	0	+	+	+
	1	–	+	+
	22	+	+	+
	23	+	+	+
512B	0	+	+	+
	1	+	+	+
	19	+	+	+
	20	+	+	+
514B	0	+	+	+
	1	–	+	+
	21	–	+	+
	22	–	+	+

†Samples were obtained from calves before suckling on day of birth (day 0), at 1 day of age, and at approximately 3-week intervals thereafter until a classification was assigned. For the 3-week time point, samples were obtained on 2 consecutive days.
See Figure 1 for remainder of key.

Table 1—Results* of virus isolation for bovine viral diarrhea virus (BVDV) from control heifers

Heifer	Buffy coat cells: Day 4	Day 5	Day 6	Day 7	Day 8	Serum: Day 2	Day 3	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
476	–	–	+	+	+	–	–	–	+	–	–	–	–
478	–	–	+	+	+	–	–	–	+	–	–	–	–
484	–	–	+	+	+	–	–	–	+	+	–	–	–
485	–	–	–	+	+	–	–	–	+	+	–	–	–
496	–	–	–	+	+	–	–	–	+	–	–	–	–
497	–	–	–	–	+	–	–	–	–	+	–	–	–
499	–	–	+	+	+	–	–	–	+	+	–	–	–
503	–	–	–	+	+	–	–	–	+	+	–	–	–
507	–	–	–	–	–	–	–	–	+	+	–	–	–
512	–	–	+	+	+	–	–	–	+	+	–	–	–
514	–	–	+	+	+	–	–	–	+	+	–	–	–

*For each sample type, 4 wells were inoculated with undiluted sample, and 4 wells were inoculated with diluted sample (dilution, 1:10). Results were scored as positive (+) when ≥ 1 well (diluted or undiluted) had fluorescence and negative (–) when none of the wells had fluorescence.
Day 0 = Day of challenge exposure.

after challenge exposure, but none of the vaccinated heifers were leukopenic. Significant differences were found between WBC counts for control and vaccinated heifers on days 2, 6, and 10 after challenge exposure.

Virus isolation from heifers after challenge exposure—Virus isolation was attempted on buffy coat cells obtained from the heifers on days 4 to 8 after challenge exposure and on serum samples obtained on days 2, 3, and 5 to 10 after challenge exposure. We isolated BVDV from buffy coat cells obtained from 10 of 11 control heifers and from serum samples of all control heifers. Virus was initially isolated from buffy coat cells obtained on day 6 after challenge exposure, and 10 of 11 samples obtained on day 8 after challenge exposure yielded positive results. Virus was detected in serum samples obtained on days 6 and 7 after challenge exposure, but all heifers had negative results for samples obtained on day 8 after challenge exposure (**Table 1**). Virus was not detected in buffy coat cells or serum samples of any vaccinated heifers.

Parturition—Of the 35 heifers that were confirmed pregnant, all gave birth to calves. Thirty-six calves were born to the 35 heifers enrolled in the study (1 of the heifers vaccinated SC gave birth to twins). Physical examination of calves on the day of birth did not indicate evidence of congenital defects in any calf. In addition, body weight of each calf was within the reference range (data not shown). On the days of birth, 30 of 36 calves had BVDV SN titers of < 1:2, and 6 had BVDV SN titers of 1:2 (calves born to 2 control and 4 vaccinated heifers).

Virus isolation from calves—All 11 control calves were positive for BVDV prior to colostrum ingestion on the day of birth. Virus was isolated from serum, nasal-swab specimens, and buffy coat samples obtained from 10 of 11 calves born to control heifers and from serum and nasal-swab specimens obtained from the other calf born to a control heifer. All calves born to control heifers were also positive for BVDV at 1 day of age. However, fewer wells had positive results at 1 day of age than on the day of birth; this could have been attributable to neutralization of BVDV by colostral antibodies. All calves born to control heifers were also positive for BVDV at 21 days of age. Virus was isolated from all 3 types of samples (ie, serum, nasal-swab specimens, and buffy coat samples) for 7 of 11 calves born to control heifers and from 2 types of samples for the other 4 calves born to control heifers (**Table 2**). In most virus isolations from calves born to control heifers, the samples were strongly positive (ie, all wells had positive results for fluorescence in the undiluted samples and the 1:10 dilution). All calves born to control heifers were classified as persistently infected (Fig 1).

Bovine viral diarrhea virus was isolated from 1 of 11 calves born to heifers vaccinated IM. That calf had positive results on the day of birth and at 1 day of age, but results of virus isolation were negative at 21, 22, and 45 days of age (**Table 3**); thus, it was classified as not being persistently infected. Therefore, all calves from heifers vaccinated IM were classified as not being persistently infected. Frequency of persistently infected calves born to heifers vaccinated IM and those born to control heifers differed significantly ($P = 0.01$).

Of 14 calves born to heifers vaccinated SC, includ-

Table 3—Results* of virus isolations for BVDV from 5 calves born to heifers vaccinated IM or SC with a BVDV vaccine

Route of vaccination	Calf	Day of age†	Virus isolation: Serum	Virus isolation: Nasal-swab specimen	Virus isolation: Buffy coat cells	Classification
IM	477B	0	–	+	+	Not persistently infected
		1	+	+	–	
		21	–	–	–	
		22	–	–	–	
		45	–	–	–	
SC	481B	0	+	–	+	Persistently infected
		1	–	–	–	
		22	+	+	–	
		23	+	–	–	
		44	–	–	–	
SC	498B	0	–	+	–	Persistently infected
		1	–	+	–	
		20	–	–	–	
		21	–	–	–	
		49	–	+	–	
		71	–	–	–	
SC	505B	0	–	–	–	Not persistently infected
		1	–	–	–	
		21	–	+	–	
		22	–	–	–	
		56	–	–	–	
		78	–	–	–	
SC	509B	0	–	+	+	Not persistently infected
		1	+	+	+	
		21	–	–	–	
		22	–	–	–	
		45	–	–	–	

See Tables 1 and 2 for key.

ing twins born to 1 heifer, BVDV was isolated from 4 at least 1 time. One calf was positive for BVDV on the day of birth and at 22 and 23 days of age, but it had negative results at 1 and 44 days of age. A second calf was positive on the day of birth and at 1 and 49 days of age, but it had negative results at 20, 21, and 71 days of age. A third calf was positive in 1 type of sample at 21 days of age, but it had negative results on all other test days. A fourth calf was positive on the day of birth and at 1 day of age, but it had negative results on all other test days (Table 3). Interestingly, only 2 samples obtained from calves born to heifers vaccinated SC were strongly positive; most samples yielded positive results for fluorescence in less than half of the wells. Also, only 1 calf on 1 testing day had positive results for all 3 types of samples; most of the isolations were from nasal-swab specimens.

One calf born to a heifer vaccinated SC was found dead at 3 days of age. Necropsy revealed that the calf was dehydrated and that there was a small amount of hemorrhage and congestion in the lungs. Death was at least partially attributable to extremely hot ambient temperatures (32 to 38°C) on the 2 preceding days. Because this calf died before it reached 21 days of age, tissues were collected during necropsy and tested for BVDV. This calf had negative results for BVDV in nasal-swab specimens, serum samples, and buffy coat samples obtained on the day of birth and at 1 day of age, and tissues from the calf obtained during necropsy all had negative results for BVDV (data not shown). Because BVDV was not isolated from samples obtained before this calf suckled colostrum, and BVDV was not detected in any tissue samples obtained during necropsy, this calf was classified as not persistently infected.

On the basis of the previously established diagnostic scheme (Fig 1), only 2 calves born to heifers vaccinated SC were classified as persistently infected. All tissues obtained from these 2 calves during necropsy yielded negative results for BVDV (data not shown). Assuming that both of these calves were, in fact, persistently infected, the prevalence of persistently infected calves born to heifers vaccinated SC and those born to control heifers differed significantly ($P = 0.001$). However, the prevalence of persistently infected calves born to heifers vaccinated IM and heifers vaccinated SC did not differ significantly ($P = 0.482$).

Discussion

A single vaccination with BVDV1 strain WRL caused seroconversion to BVDV by the first sample collection after vaccination, and antibody titers continued to increase until the day of challenge exposure, whereas control heifers remained seronegative during the same period. The true duration of immunity was not examined, but it was documented in another study[21] that the duration of immunity of the same vaccine against BVDV2 challenge was at least 7 months after a single vaccination. The regimen used in the study reported here (ie, vaccination 21 days prior to the start of breeding) is consistent with recommended management practices.[2,22] Breeding was by natural means, and 36 of 40 (90%) heifers were confirmed pregnant.

The BVDV strain 7443 that was used for challenge exposure was originally isolated from a persistently infected bull.[23,24] The study reported here was designed to examine protection against persistent infection of offspring as a result of vaccination; it was not designed to examine protection against abortion. Therefore, timing of the challenge exposure and the challenge strain of BVDV selected were optimal for achieving this objective. The BVDV strain 7443 readily established viremia in all unvaccinated control heifers. The IV route of inoculation was selected because of the fact it was most likely to result in transmission of the virus to the fetus. Under the premise that viremia is required for transmission of BVDV to the fetus, serum and buffy coat samples of heifers were tested for BVDV. All control heifers were viremic. In addition, leukopenia rapidly developed in 7 control heifers 2 days after IV inoculation of the challenge virus, suggesting infection of a large percentage of the circulating leukocytes in the control heifers. In contrast, none of the vaccinated heifers were viremic, and leukopenia was not detected in the vaccinated heifers. The rapid clearance of virus was consistent with high neutralizing-antibody titers in the vaccinated heifers at the time of challenge exposure.[22]

Clinical signs of disease (nasal discharge and mild diarrhea) were sporadically observed in heifers from all 3 groups. These signs are difficult to interpret in the vaccinated heifers in light of the lack of viremia or leukopenia in those heifers. Several heifers had serous nasal discharge prior to challenge exposure, possibly in response to fluctuating environmental conditions at the time of challenge inoculation. In addition, the stress of daily handling of such large cattle during midgestation may have exacerbated some of the observed clinical signs. Bovine viral diarrhea virus is found in nasal secretions, circulating lymphocytes, and serum in persistent and acute BVDV infections. Therefore, it is not possible to conclusively identify a persistently infected calf on the basis of a single positive virus isolation. Rather, to classify a calf as persistently infected with BVDV, it must have positive results when tested for BVDV in 2 samples obtained at least 21 days apart, which is generally believed to allow sufficient time for the virus to be cleared from an acutely infected animal.[25,26] However, recurrent infections have been described in which BVDV was detectable 21 days or more following experimental infection with cytopathic[27] and noncytopathic[28] biotypes. This could have resulted in misclassification of persistently infected calves in our study. However, because of the temporal pattern of positive results, the proportion of calves with positive results, and the proportion of tissues with positive results, we considered our definition of persistent infection to be valid. Furthermore, these results documented a marked sparing effect of vaccination on frequency, pattern, and proportion of BVDV detection.

Neutralizing antibodies against BVDV in the circulation may interfere with the ability to isolate BVDV from a persistently infected calf.[29] This occurs once a calf has ingested colostrum from a seropositive dam. For this reason, it is critical to obtain samples from calves prior to suckling. Therefore, to conclusively identify a calf as not persistently infected, it must have negative results for virus isolation on 2 samples

obtained at least 21 days apart. These principles formed the basis for the classification scheme used in the study reported here. The limited number of virus isolations that yielded positive results on samples obtained from calves born to vaccinated dams was likely attributable to acute infection or cross-contamination caused by the large number of persistently infected calves on the premises during the sample collection period.

Consistent with the observation of viremia in all control heifers, BVDV1 strain 7443 apparently spread readily to the fetus and established persistent infection in 100% of calves born to unvaccinated heifers. None of the calves from heifers vaccinated IM were persistently infected, and only 2 of 14 calves from heifers vaccinated SC were classified as persistently infected. The pattern of results for virus isolation in the BVDV-positive calves born to vaccinated heifers differed from the pattern in most BVDV-positive calves born to control heifers. Bovine viral diarrhea virus was readily isolated from undiluted samples and 1:10 dilutions of serum, buffy coat cells, and nasal-swab specimens obtained from calves born to control heifers. In contrast, most isolations of BVDV from calves born to vaccinated heifers were from nasal-swab specimens and yielded positive results in less than half of the inoculated cultures. Furthermore, BVDV was not detected in tissues of the 2 persistently infected calves born to vaccinated heifers. Alternatively, as mentioned previously, positive results for virus isolation may have been attributable to an acute infection that was misclassified as a persistent infection. In either case, the most conservative classification is to assume that the 2 calves born to vaccinated heifers were, in fact, persistently infected. However, analysis of the data suggests that the transmission of BVDV from calves born to vaccinated dams would likely be much lower than from calves born to control heifers.

Overall, we did not detect significant differences in the proportions of persistently infected calves born to heifers vaccinated IM or SC. Vaccination provided protection against persistent infection of at least 92% of fetuses, and this amount of protection differed significantly for each route of vaccination, compared with values for the control heifers.

A major concern among bovine veterinarians is the antigenic diversity of BVDV isolates. In this study, the BVDV1 WRL vaccine was efficacious against the heterologous and antigenically distinct BVDV1 strain 7443 that was used for challenge inoculation. Some field strains of BVDV2 that have emerged in cattle populations appear to share limited serologic cross-reactivity with BVDV1 vaccine strains.[4-7] The BVDV1 WRL vaccine prevents viremia and clinical disease following challenge with BVDV2 strain 890 in an acute infection model.[21] Additional investigations will be required to determine whether BVDV1 WRL vaccine is also effective in reducing or preventing fetal infection with BVDV2. The effectiveness of the BVDV1 WRL vaccine against a spectrum of antigenically distinct BVDV isolates is likely attributable to the high antibody titers elicited by the vaccine, which confer sufficient cross-reactivity against heterologous strains tested.

Data documenting efficacy of licensed or experimental BVDV vaccines in preventing fetal infection are limited. In 2 studies[30,31] that involved the use of inactivated vaccines, protection was incomplete. Conversely, 1 study[32] did include results of heifers vaccinated with 2 or 3 doses of inactivated BVDV1 vaccine followed by challenge exposure of vaccinated and control heifers with a BVDV1 strain. In that study, abortion or birth of persistently infected calves was reported in 5 of 6 unvaccinated control heifers, but BVDV was not isolated from 13 live calves and 2 aborted fetuses from vaccinated heifers. Relationship of the vaccine strain to the challenge strain or efficacy of the inactivated vaccine against BVDV2 isolates was not reported. In another study,[33] investigators reported protection against persistent infection for 10 of 12 calves born to heifers vaccinated with an MLV BVDV1 vaccine followed by heterologous challenge exposure, whereas persistently infected calves were born to all 6 unvaccinated heifers. Results of another study[34] revealed that partial protection against persistent infection of the fetus was provided by prebreeding vaccination of 19 heifers or cows with a BVDV1 vaccine that were challenge exposed to BVDV2 at 75 days of gestation. In that study, 6 of 6 fetuses from unvaccinated cows and 8 of 19 fetuses from vaccinated cows were persistently infected. Generally, results of studies reported in the literature suggest that some degree of fetal protection can be achieved through vaccination of dams with some MLV BVDV vaccines before breeding.

Persistently infected cattle are the primary reservoir of BVDV in the cattle population.[1,2] The key to controlling the transmission of BVDV is prevention of fetal infection. Field isolates are antigenically variable, presenting a challenge to development of vaccines and to reduction of virus transmission in the cattle population.[35] Results of the study reported here document the feasibility of prevention of viremia and virus transmission of BVDV1 to fetuses. Results of another study[21] that involved the use of this vaccine strain suggest that fetal protection against strains of BVDV2 is also likely, although the studies necessary to confirm this supposition have not yet been performed.

[a]BVDV FITC conjugate, VMRD, Pullman, Wash.
[b]BME, Gibco, Rockville, Md.
[c]Bovine serum, Harmon Technological, Gowrie, Iowa.
[d]SST vacutainer, Becton, Dickinson & Co, Franklin Lakes, NJ.
[e]Falcon sterile fiber-tipped applicators, Becton, Dickinson & Co, Franklin Lakes, NJ.
[f]Neomycin sulfate, Gibco, Rockville, Md.
[g]Gentacin, Gibco, Rockville, Md.
[h]Penicillin-streptomycin, Gibco, Rockville, Md.
[i]Penicillin-streptomycin, Gibco, Rockville, Md.
[j]Bacitracin, Sigma Chemical Co, St Louis, Mo.
[k]Heparin vacutainer, Becton, Dickinson & Co, Franklin Lakes, NJ.
[l]Histopaque-1077, Sigma Chemical Co, St Louis, Mo.
[m]Silicon dioxide, Sigma Chemical Co, St Louis, Mo.
[n]SAS, version 6.22, SAS Institute Inc, Cary, NC.

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