Fetal protection against bovine viral diarrhea virus types 1 and 2 after the use of a modified-live virus vaccine

Wenzhi Xue, Debra Mattick, Linda Smith, Jon Maxwell

Abstract

The objective of this study was to demonstrate the efficacy of a modified-live virus (MLV) vaccine in protecting fetuses from infection with type 1 or type 2 *Bovine viral diarrhea virus* (BVDV) when pregnant heifers were challenged at approximately 170 d of gestation with noncytopathic field isolates. The 83 pregnant heifers had been bred naturally 4 wk after vaccination. Fetuses were collected 60 d after BVDV type 2 challenge, and newborn calves were collected before colostrum intake after BVDV type 1 challenge. Protection was determined by measuring the serum neutralizing (SN) antibody response in the fetus or calf and by virus isolation from thymus, lung, spleen, and kidney tissue samples. There was a measurable SN antibody response to BVDV in all the fetuses and calves of the control heifers, which had received a placebo vaccine. However, only 4 of 22 calves and 7 of the 28 fetuses of the MLV-vaccinated heifers demonstrated SN antibody after BVDV challenge. Type 1 BVDV was isolated from tissue samples of 5 of the 12 calves of control heifers and none of 22 calves of the MLV-vaccinated heifers challenged with type 1 BVDV. Type 2 BVDV was isolated from tissue samples of 17 of the 18 fetuses of the control heifers and 2 of the 28 fetuses of the MLV-vaccinated heifers challenged with type 2 BVDV. The results of this study demonstrate that the MLV vaccine reduces the fetal infection rate by at least 82% for BVDV type 1 and by 75% for BVDV type 2 when heifers are exposed to highly fetotrophic BVDV at 170 d of gestation.

Résumé

Cette étude visait à démontrer l'efficacité d'un vaccin à virus vivant modifié (MLV) à protéger les fœtus envers l'infection par les virus de type 1 ou type 2 du virus de la diarrhée virale bovine (BVDV) lorsque des taures étaient infectées à environ 170 jours de gestation avec des isolats de champ non-cytopathogènes. Les 83 taures en gestation avaient été saillies naturellement 4 semaines après la vaccination. Les fœtus ont été récoltés 60 j après l'infection défi par le BVDV type 2 et les veaux nouveau-nés ont été récoltés avant la prise de colostrum lors de l'infection défi par le BVDV type 1. La protection était déterminée par mesure de la réponse en anticorps sériques neutralisant (SN) chez les fœtus ou les veaux, et par isolement viral à partir d'échantillons de thymus, poumon, rate et rein. Une réponse mesurable en anticorps SN envers le BVDV a été notée chez tous les fœtus et veaux des taures témoins qui avaient reçu un vaccin placebo. Toutefois, seulement 4 des 22 veaux et 7 des 28 fœtus des taures vaccinées avec le MLV ont présenté des anticorps SN contre le BVDV après l'infection défi. Le BVDV type 1 a été isolé à partir d'échantillons de tissu de 5 des 12 veaux des taures témoins et d'aucun des 22 veaux des taures vaccinés avec le MLV qui avaient été infectées avec le BVDV de type 1. Le type 2 du BVDV a été isolé à partir des échantillons de tissu de 17 des 18 fœtus des taures témoins et de 2 des 28 fœtus des fœtus des taures vaccinées avec le MLV et infectées avec le type 2 de BVDV. Les résultats de cette étude démontrent que le vaccin MLV réduit le taux d'infection fœtales par au moins 82 % pour le BVDV type 1 et de 75 % pour le BVDV type 2 lorsque les taures sont exposées à du BVDV ayant un fort tropisme pour les fœtus à 170 jours de gestation.

(Traduit par Docteur Serge Messier)

Introduction

Bovine viral diarrhea virus (BVDV), a pestivirus of the family *Flaviviridae,* is an important pathogen for the cattle industry, often resulting in severe economic losses (1,2). Disease associated with BVDV can range from clinically inapparent to severe and can involve the respiratory, enteric, reproductive, immune, and endocrine systems (3–5). Infection with BVDV poses a major threat to the cattle industry even though vaccines are commercially available. Although vaccines targeting respiratory diseases have been the primary interest of cattle producers, the focus has shifted to include reproductive efficacy, mainly fetal protection, owing to the increase in BVDV-related reproductive losses in the United States (6). Intrauterine infection, often resulting in reproductive dysfunction, is the primary reproductive impact of BVDV (7,8). The virus is able to infect the female genital tract, cross the placenta, and thus infect the fetus (7). If infection by a noncytopathic (ncp) BVDV biotype occurs in the early stage of gestation (approximately 30 to 90 d), the calf will be born persistently infected (PI), because the fetus does not have a fully developed immune system at the time of infection (7–9). The PI animals, a population of less than 1% in cattle herds, are a major concern for cattle producers because they are the primary source for

Biological Research and Development, Intervet/Schering Plough Animal Health, 35500 West 91st Street, De Soto, Kansas 66018, USA.
Address all correspondence to Dr. Wenzhi Xue; telephone: (913) 422-6889; fax: (913) 422-6074; e-mail: wenzhi.xue@sp.intervet.com
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spread of BVDV within and among cattle herds (10–12). Such calves continually shed large amounts of BVDV and thus pose a risk to susceptible herdmates. After the 1st trimester (from 90 d to the end of gestation), the fetus is immunocompetent, and BVDV infection can induce the production of serum neutralizing (SN) antibodies (7,13,14). However, BVDV infection of fetuses at this stage often results in abortion or nonviability due to a number of congenital abnormalities (7,14).

A successful prevention and control strategy for BVDV depends upon the prevention of fetal infections by proper vaccination and removal of PI animals from herds. Previous studies have demonstrated that vaccination of heifers before breeding reduces the rate of or prevents persistent infection of their offspring (15–22). Nearly all of these studies conducted the BVDV challenge in the early stages of gestation. Only 1 study, which used an inactivated-virus vaccine, reported fetal protection when dams were challenged in the late stage of gestation by continuous exposure to animals with persistent BVDV infection (22). The objective of the study reported here was to determine the efficacy of a commercially available multivalent BVDV vaccine containing attenuated live BVDV types 1 and 2 in terms of fetal protection after challenge of the dams with either BVDV type 1 or type 2 at about 170 d of gestation.

Materials and methods

Animals and vaccination

A total of 144 nonpregnant beef heifers of breeding age were included in 2 studies: BVDV type 1 challenge and BVDV type 2 challenge. Before vaccination, all animals were determined to be seronegative for both BVDV types, with reciprocal SN antibody titers of less than 1:2. The heifers and bulls for breeding were confirmed to be free of persistent BVDV infection by Iowa State University Diagnostic Laboratory, Ames, Iowa, USA.

For the BVDV type 1 challenge, 61 heifers were randomly divided into vaccinated and control groups: 41 were vaccinated subcutaneously with a single dose (2 mL) of a commercial modified-live virus (MLV) vaccine (Vista 5 SQ; Intervet, Millsboro, Delaware, USA) containing BVDV types 1 and 2, *Infectious bovine rhinotracheitis virus, Bovine parainfluenza virus 3,* and *Bovine respiratory syncytial virus;* the other 20 received a placebo vaccine. After breeding, pregnancy was confirmed for 37 heifers (25 in the MLV-vaccinated group and 12 in the control group), which were subsequently challenged with a BVDV type 1 ncp strain.

For the BVDV type 2 challenge, 83 heifers were randomly divided into vaccinated and control groups: 55 were vaccinated subcutaneously with the commercial vaccine, and the other 28 received the placebo vaccine. After breeding, pregnancy was confirmed for 46 heifers (28 in the MLV-vaccinated group and 18 in the control group), which were subsequently challenged with a BVDV type 2 ncp strain.

Synchronization and breeding

On the day of vaccination, estrus synchronization of the heifers was conducted with a standard protocol. Four weeks after vaccination, the heifers were commingled with bulls for 2 wk. With the use of ultrasonography, pregnancy was determined 7 wk after removal of the bulls. The pregnant heifers were subsequently palpated twice for pregnancy confirmation during the study at 6 and 12 wk after the initial pregnancy check.

Challenge with field isolates

At about 170 (163 to 177) d of gestation, the pregnant heifers were intranasally challenged with an aerosolized ncp BVDV type 1 or type 2 field isolate by means of a DeVilbiss Atomizer (Sommerset, Pennsylvania, USA). The challenge viruses, kindly provided by the Diagnostic Laboratories, South Dakota State University, Brookings, South Dakota, USA, had been isolated from aborted fetuses submitted from field cases (herd histories were unknown), then identified and genotyped. The 37 heifers challenged with BVDV type 1 strain SD02 BVD9 each received 2.5×10^6 $TCID_{50}$ (50% tissue culture infectious dose) of virus. The 46 heifers challenged with BVDV type 2 strain SD02 BVD5 each received 1.0×10^6 $TCID_{50}$ of virus.

Sample collection

Blood samples were collected from the heifers on the days of vaccination (day 0), breeding (day 28), and challenge (day 205), as well as 2 wk after challenge (day 219), to determine titers of SN antibody against BVDV types 1 and 2. Additionally, on day 0 and days 5 to 10 after challenge (study days 205 and 210 to 215), the buffy coat (the concentrated layer of leukocytes between the plasma and the erythrocytes) was collected for virus isolation to determine whether challenge had caused viremia in the heifers.

After the BVDV type 1 challenge, blood samples were taken from newborn calves before any colostrum intake. The calves were then euthanized, and tissue samples were taken from the thymus, lung, spleen, and kidney for virus isolation. Serum was analyzed to determine SN antibody titers and IgG content; samples with an IgG content of 800 mg/dL or greater were excluded from further analysis, as it was presumed that the calves had ingested colostrum before sample collection.

After the BVDV type 2 challenge the heifers were euthanized at about 60 d (study day 265) and the fetuses harvested. Blood samples were collected from each fetus, and the serum was tested for SN antibody to BVDV. Buffy coats, as well as tissue samples from the thymus, lung, spleen, and kidney, were obtained for virus isolation.

Virus isolation

The heparinized blood samples were centrifuged at $500 \times g$ for 20 min. From each sample, the buffy coat was collected by means of a Pasteur pipette. The buffy coats were washed by centrifugation in 5 mL of Dulbecco's Modified Eagle Medium (DMEM), and then the pellets were resuspended in 2 mL of DMEM. Monolayers of Madin–Darby bovine kidney (MDBK) cells in 24-well tissue culture plates were used for virus isolation. A 0.5-mL aliquot of buffy coat was added to each of 2 wells per sample. The plates were incubated for 1 h at 37°C (with 5% CO_2 supply) for adsorption and then rinsed twice with DMEM supplemented with 10% horse serum. Next, 2 mL of DMEM with 10% horse serum was added to each well, and the plates were incubated at 37°C for 3 d. The plates were frozen at -70°C for 2 h and then thawed, after which 0.5 mL of culture fluid from each well was used for a 2nd passage on fresh cells. After adsorption the plates were not rinsed, and 1.5 mL of DMEM with 10% horse

Table I. Mean reciprocal titers of serum neutralizing (SN) antibody to types 1 and 2 *Bovine viral diarrhea virus* (BVDV) in heifers before and after vaccination (on day 0) with a modified-live virus vaccine or a placebo, breeding (on day 28), and intranasal challenge with a noncytopathic field isolate of BVDV (on day 205)

	BVDV type to which SN antibody was demonstrated; titer on various days of the study							
BVDV field isolate type	Type 1				Type 2			
and heifer group	0	28	205	219	0	28	205	219
Type 1								
Vaccinated (n = 25)	0	1877	1058	4403	0	90	89	282
Control (n = 12)	0	0	0	17	0	0	0	5
Type 2								
Vaccinated (n = 28)	0	1910	985	3835	0	27	67	746
Control (n = 18)	0	0	0	4	0	0	0	16

serum was added to each well. The plates were then incubated for 3 more days at 37°C, frozen at -70°C for 2 h, and thawed. Again, 0.5 mL of culture fluid was used for a passage on fresh cells, with the same procedure as for the 2nd passage. After 3 d of incubation the plates were fixed with 80% acetone and processed for the detection of immunofluorescent viral antigen, by means of a fluorescence microscope, with the use of BVDV type-specific monoclonal antibodies.

Each tissue sample from a fetus or newborn calf was minced in a large Petri dish with a disposable sterile scalpel. The minced tissue was placed in a Whirlpak bag that contained 3 mL of DMEM with 1x neomycin and macerated for 1 min with a stomacher. Finally, the homogenized tissue samples were transferred to a 24-well tissue culture plate containing MDBK monolayers. Three passages were conducted, according to the virus isolation procedure described for buffy coats.

Statistical analysis

The results for viremia in the heifers, BVDV SN antibody titers in the newborn calves or fetuses, and virus isolation from fetal tissue samples were analyzed and comparisons made between the MLV-vaccinated and control groups, with the use of PROC NPARWAY, version 9.1.3, of SAS (version 8.0; SAS Institute, Cary, North Carolina, USA) to perform the Fisher exact test.

Results

Serologic response to vaccination

The reciprocal titers of SN antibody to BVDV types 1 and 2 in the heifers before and after vaccination and after challenge are summarized in Table I. The MLV-vaccinated heifers had a strong serologic response to BVDV after vaccination, and the titers were still high at the time of BVDV challenge. The control heifers maintained a serologically negative BVDV status before challenge.

Viremia in the heifers

The extent of viremia caused by the BVDV challenge, as determined by virus isolation from buffy-coat samples, is summarized in Table II. Among the heifers challenged with BVDV type 1, virus was isolated from all 12 in the control group, the number with positive results peaking on days 5 to 8 after challenge. However, the virus was isolated from only 1 (4%) of the 25 MLV-vaccinated heifers, and only on day 6. Similarly, among the heifers challenged with BVDV type 2, virus was isolated from all 18 in the control group, the number with positive results peaking on days 5 to 10, but from only 2 (7%) of the 28 MLV-vaccinated heifers, and only on day 6 after challenge.

Antibody development in the fetuses

Bovine fetuses can produce SN antibody to BVDV after 120 d of gestation. Therefore, development of such antibody in the fetus when the heifer was challenged at about 170 d of gestation would be a reliable indicator of fetal infection. After the BVDV type 1 challenge, the heifers were maintained until calved, and the newborn calves were prohibited from suckling before blood collection. In spite of precautions, serologic analysis showed that 4 calves had an IgG concentration of 800 mg/dL or greater and therefore must have ingested colostrum; these calves were excluded from the study. The SN antibody analysis demonstrated a serologic response to BVDV type 1 in all 11 remaining calves born to the control heifers, the titers ranging from 1:96 to 1:1024, but in only 4 (18%) of the remaining 22 calves born to the MLV-vaccinated heifers, the titers ranging from 1:24 to 1:128. The difference between the 2 groups of calves was significant ($P < 0.01$).

To ensure complete colostrum deprivation after the BVDV type 2 challenge, the pregnant heifers were euthanized before calving (2 mo after challenge), and blood samples were collected from the fetuses. The SN antibody analysis demonstrated a serologic response to BVDV type 2 in all 18 of the fetuses from the control heifers, the titers ranging from 1:12 to 1:256, but in only 7 (25%) of the 28 fetuses from the MLV-vaccinated heifers. The difference between the 2 groups of calves was again significant ($P < 0.01$).

These results demonstrate reductions in the incidence of fetal BVDV infection of 82% and 75% when MLV-vaccinated heifers were challenged with BVDV type 1 and type 2, respectively, at about 170 d of gestation.

Table II. Isolation of BVDV from buffy-coat samples from the heifers after BVDV challenge

BVDV field isolate type and heifer group	Positive results: Day after challenge; Number of heifers							
	0	5	6	7	8	9	10	%
Type 1								
Vaccinated (n = 25)	0	0	1	0	0	0	0	4
Control (n = 12)	0	10	10	8	7	3	0	100
Type 2								
Vaccinated (n = 28)	0	0	2	0	0	0	0	7
Control (n = 18)	0	16	17	15	13	7	1	100

Table III. Isolation of BVDV from tissue or buffy-coat samples from the newborn calves or fetuses after BVDV challenge of the heifers

BVDV field isolate type and heifer group	Positive results: Sample type; Number of calves or fetuses					Total no. (and %)
	Thymus	Lung	Spleen	Kidney	Buffy coat	
Type 1						
Vaccinated (n = 25)	0	0	0	0	NA	0 (0)
Control (n = 12)	0	3	3	2	NA	5 (42)
Type 2						
Vaccinated (n = 28)	1	1	1	0	2	2 (7)
Control (n = 18)	10	10	11	11	12	17 (94)

NA — not applicable.

Virus isolation from fetal samples

Virus isolation from tissue samples is another way of identifying fetal infection by BVDV. The results in this study are summarized in Table III.

After the BVDV type 1 challenge, virus was isolated from thymus, lung, spleen, and kidney tissue samples taken from the newborn calves of 5 (42%) of the 12 control heifers but none of the 25 MLV-vaccinated heifers.

After the BVDV type 2 challenge, virus was isolated from thymus, lung, spleen, and kidney tissue samples taken from 17 (94%) of the 18 fetuses of the control heifers, as well as from the buffy coats of 5 (28%) of the 18 control fetuses, but from only 2 (7%) of the 28 fetuses of the MLV-vaccinated heifers. The difference between the 2 groups of fetuses was significant ($P < 0.01$).

Discussion

In addition to being a primary pathogen in the bovine respiratory disease complex, BVDV is a major pathogen in reproductive diseases of cattle, especially by way of fetal infection (13,14,17–24). Prevention of fetal infection has become a standard criterion for efficacy of BVDV vaccination. Numerous studies have demonstrated the efficacy of BVDV vaccines in preventing persistent infection with BVDV (15–22); however, this is the 1st study to demonstrate the efficacy of an MLV vaccine in preventing fetal infection when the heifers were challenged in the later stages of gestation.

Transplacental infection of the developing fetus can occur in susceptible cows with BVDV. The outcome largely depends upon the time of infection during pregnancy, the immunocompetence of the developing fetus, the BVDV biotype, and the virulence of the virus (7). After infection of pregnant heifers, BVDV crosses the placenta and can begin to replicate in the fetus within 1 wk (13,14). Before 120 d of gestation, infection of the fetus with ncp BVDV usually results in a PI fetus (7,25). Noncytopathic BVDV is the only biotype that has been observed clinically or experimentally to cause persistent infection with BVDV (7,23,25). After 120 d of gestation, fetuses become immunocompetent and respond to viral infection, although abortion can still be caused by BVDV infection at this time (7). The development of SN antibody to BVDV in fetuses is a strong indicator that the fetuses were infected by BVDV. One study showed that challenge of unvaccinated dams with ncp BVDV strains produced fetal infection in 100% of animals (26).

We challenged pregnant cows at about 170 d of gestation with either type 1 or type 2 ncp BVDV strains that had been isolated from aborted fetuses. After challenge, virus was isolated from buffy-coat samples from all the control heifers, but only a few MLV-vaccinated heifers showed viremia, which is commonly considered a precursor to fetal infection. Prevention of viremia in pregnant heifers by vaccination likely indicates the prevention of fetal infection as well. When the fetuses were infected by BVDV near 170 d of gestation, SN antibody was induced. Therefore, the development of SN antibody in response to BVDV in newborn calves before colostrum intake or in fetuses was the most important factor in defining fetal infection in this study. All the offspring of the control heifers produced SN antibody against BVDV in each challenge, indicating 100% fetal infection by the challenging BVDV strains. In contrast, only 4 (18%) of 22 newborn calves of MLV-vaccinated heifers were SN-antibody-positive after challenge with BVDV type 1. Similarly, only 7 (25%)

of 28 fetuses of MLV-vaccinated heifers were SN-antibody-positive after challenge with BVDV type 2. The incidence of fetal BVDV infection was therefore reduced by 82% and 75% when MLV-vaccinated heifers were challenged with BVDV type 1 and type 2, respectively, at about 170 d of gestation.

Virus isolation from fetal samples is another indicator of fetal infection by BVDV. In this study, virus was isolated more frequently from fetuses after challenge with BVDV type 2 than from newborn calves after challenge with BVDV type 1. This difference may be partially due to the fact that BVDV type 2 has a higher titer in blood than BVDV type 1, as reported by others (22,29). Another reason may be that once the SN antibody has developed in infected fetuses, the clearing of virus from infected tissues is gradual. Therefore, many newborn calves and fetuses had demonstrable SN antibody against BVDV, but their tissues did not yield the virus. Because BVDV infection of pregnant cows often results in abortion and other reproductive problems, fetal protection by vaccination of heifers before breeding is always beneficial to cattle producers and especially on dairy farms.

In our previous studies, the MLV vaccine used in this study, when vaccinated pregnant heifers were challenged in the 1st trimester pregnancy, demonstrated more than 96% and 91% protection against fetal persistent infection with BVDV type 1 and type 2, respectively (unpublished data), rates similar to those seen in other vaccination studies (15–21). In this study, compared with infection in control fetuses, the incidence of fetal infection with BVDV type 1 and type 2 was reduced by 82% and 75%, respectively, in the MLV-vaccinated heifers. These significant reductions suggest that the MLV vaccine is successful in preventing BVDV in pregnant heifers exposed late in pregnancy, as well as in providing fetal protection, when administered 4 wk before breeding. Fetal protection by a killed BVDV vaccine when vaccinated pregnant heifers were challenged late in pregnancy has been reported (22). However, this study is the first study to demonstrate that an MLV BVDV vaccine protects against fetal infection when the dams are challenged beyond the 1st trimester (at about 170 d of gestation).

The BVDV challenge strains used in this study were isolated from aborted fetuses in the field and are heterologous with the strain in the MLV vaccine BVDV. As a result, protection greater than 75% against the challenge viruses demonstrates the strong efficacy of this vaccine against BVDV types 1 and 2.

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