Protection against Fetal Infection with Either Bovine Viral Diarrhea Virus Type 1 or Type 2 Using a Noncytopathic Type 1 Modified-Live Virus Vaccine*

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\textbf{INTRODUCTION}

Bovine viral diarrhea virus (BVDV) infection is a constant concern to the cattle industry. Infection of cattle with BVDV can result in a wide spectrum of clinical signs that can vary from inapparent infection to mild fever, diarrhea, depression, and death. Reproductive failure, leukopenia, respiratory and gastrointestinal problems, generalized immunosuppression, and increased susceptibility to secondary bacterial infections are also associated with BVDV infections.\textsuperscript{1}

\textbf{CLINICAL RELEVANCE}

Two bovine viral diarrhea virus (BVDV) fetal protection studies were done using a monovalent noncytopathic (NCP) BVDV vaccine containing type 1 BVDV. In study 1, thirty-two fetuses (23 vaccinates and nine controls) were recovered following fetal challenge with the type 1a BJ strain. Twenty of 23 fetuses from the vaccinates were negative for BVDV type 1, but all of the controls (nine of nine) were infected. In study 2, 22 animals (14 vaccinates and eight controls) were challenged with the type 2 PA131 strain. Thirteen of the 14 fetuses from the vaccinates were negative for BVDV type 2, but all of the nonvaccinated controls (eight of eight) were infected. These results indicate the efficacy of a monovalent NCP BVDV vaccine in providing excellent protection against either BVDV type 1 or type 2 fetal infection.

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BVDV is a positive-stranded RNA Pestivirus belonging to the family *Flaviviridae*. BVDV isolates can be classified into two genotypes, type 1 and type 2. BVDV can also be classified into two biotypes, noncytopathic (NCP) and cytopathic (CP). Each genotype contains both NCP and CP strains, but NCP is more prevalent in field isolates.

Acute BVDV infection of pregnant cattle generally causes subclinical disease; however, infection with an NCP strain at 40 to 150 days of gestation may result in the birth of calves that are persistently infected (PI). PI calves are immunotolerant to the infecting NCP strain. These PI calves have an enormous economic impact and pose a continual threat to other cattle through intermittent shedding of virus. Superinfection of these calves with an antigenically similar CP strain of BVDV causes a fatal mucosal disease in which the calf develops severe hemorrhagic diarrhea and thrombocytopenia.

Control and prevention of BVDV infection in a herd involves diagnostic testing, biosecurity, and vaccination. Vaccination helps protect cattle against the development of acute BVDV infection, subsequently reducing the number of PI calves born each year. In recent years, the ability of a BVDV vaccine to be cross-protective against both type 1 and type 2 genotypes has become a major concern.

This article describes two studies that were performed to evaluate the efficacy of a BVDV type 1 modified-live virus (MLV) vaccine to protect calves in utero from developing a persistent infection after either a heterologous type 1 or type 2 challenge of heifers.

**MATERIALS AND METHODS**

**Animals**

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee. Heifers in both studies were approximately 18 months of age and had serum neutralizing antibody titers of 1:5 or less to both BVDV type 1 (BJ) and BVDV type 2 (PA131). Forty-one Limousin-Angus crossbred heifers were used in the type 1 study (study 1); 38 Limousin-Angus crossbred heifers were used in the type 2 study (study 2).

**Vaccine**

Heifers in both studies were vaccinated with a monovalent MLV vaccine containing a type 1a NCP BVDV (i.e., the BVDV component from Arsenal 4.1, Novartis Animal Health) 50 to 60 days before breeding.

**Study 1**

Twenty-seven heifers were administered a single dose of vaccine in the neck region; 14 heifers were vaccinated SC, and 13 heifers were vaccinated IM. Fourteen heifers were retained as nonvaccinated controls (NVCs).

**Study 2**

Twenty-six heifers were administered a single dose of vaccine in the neck region; 13 heifers were vaccinated SC, and 13 heifers were vaccinated IM. Twelve heifers were retained as NVCs.

**Breeding**

Heifers in both studies were synchronized for estrus. Briefly, heifers were administered 2.0 ml of gonadotropin-releasing hormone (GnRH, Cystorelin, Merial) IM and a controlled internal drug release (Eazi-Breed CIDR, Pharmacia & Upjohn) progesterone implant was inserted vaginally. The CIDR implants were removed after 7 days, and the heifers were given 5.0 ml of dinoprost tromethamine (Lutalyse, Pharmacia & Upjohn) IM. Forty-eight hours later, the heifers were artificially inseminated using frozen semen from a bull that was BVDV seronegative and virus
isolation negative. Following artificial insemination, bulls that were BVDV seronegative were placed with the heifers to breed those that did not conceive through artificial insemination. Twenty-three vaccinated and 10 nonvaccinated animals in study 1 and 14 vaccinated and eight nonvaccinated animals in study 2 were bred.

Challenge

At approximately 75 days of gestation (day of challenge), all heifers were examined using ultrasonography to determine viability of the fetus by evidence of a fetal heartbeat. Animals confirmed to be pregnant were then challenged intranasally with BVDV using a DeVilbiss aerosolizer (DeVilbiss, Somerset, PA).

Study 1

Twenty-three vaccinated animals (11 IM vaccinates and 12 SC vaccinates) and 10 of the NVC animals were given an NCP type 1a BVDV (BJ) challenge of 5.0 × 10^5 cell culture infective doses_{50} (CCID_{50}) in a total volume of 5.0 ml.

Study 2

In the type 2 study, 14 vaccinated animals (six IM vaccinates and eight SC vaccinates) and eight of the NVC animals were challenged with an NCP type 2 BVDV (PA131) with an inoculum containing 5.0 × 10^5 CCID_{50} in a total volume of 5.0 ml.

Postchallenge Observations

Following experimental infection, animals in both studies were observed daily for respiratory or enteric disease and to ensure they did not develop clinical disease as evidenced by lack of appetite or depression. In addition, animals and pastures were observed for evidence of aborted fetuses or vaginal discharge.

Fetal Harvest and Tissue Collection

At approximately 150 days of gestation (75 days postchallenge), a cesarean section was performed on all pregnant heifers and the fetuses were removed from the uterus. During fetal extraction, heart blood and approximately 2 ml of amniotic fluid were obtained, and tissue samples (approximately 2 cm^2 of spleen, thymus, kidney, lung, and liver) were collected from the fetus using a scalpel and forceps. Samples were placed in individual sterile bags and processed for BVDV isolation.

Virus Isolation

All fetal tissue samples were homogenized, and aliquots of each suspension were added to T25 tissue culture flasks and incubated for 72 hours at 37°C and 5% carbon dioxide (CO_2). The flasks were freeze–thawed, and an aliquot was passaged to a new T25 tissue culture flask and maintained for 72 hours at 37°C and 5% CO_2. Aliquots from each passage were applied to 96-well plates containing BVDV-negative Madin-Darby bovine kidney (MDBK) cells for 72 hours at 37°C and 5% CO_2. The plates were then stained and examined for BVDV using a polyclonal anti-BVDV antibody that was prepared by Dr. K. V. Brock by hyperimmunizing a germ-free calf with NADL and NY-1 strains of BVDV. Aliquots of amniotic fluid and heart blood

The ability of a BVDV vaccine to be cross-protective against both type 1 and type 2 genotypes has become a major concern.
were applied to 96-well plates of MDBK cells and incubated for 72 hours at 37°C and 5% CO₂. After incubation, samples were passaged onto a new 96-well MDBK plate and incubated for 72 hours at 37°C and 5% CO₂. Plates from each passage were stained and examined for BVDV using a polyclonal anti-BVDV antibody. Positive (BVDV strain SD-1) and negative control (cells only) wells were included on each 96-well plate.

### Serology

Blood samples were collected before vaccination, on the day of challenge, and at approximately 150 days of gestation (cesarean section), and antibody titers were determined using the constant virus, decreasing serum assay with 50 to 300 fluorescent assay infectious dose (FAID₅₀/ml). The BVDV titers were determined against BJ for type 1 or PA131 for type 2.

### Statistical Analysis

The level of fetal protection (from persistent infection) was compared between routes of vaccination and between treatment groups for both studies using Fisher's exact test. Values of $P \leq .05$ were considered significant.

## RESULTS

### Postchallenge Observations

No clinical signs of BVDV were exhibited after the challenge by any of the animals in either study.

#### Serology

**Study 1**

All heifers had mean baseline BVDV type 1 antibody titers of less than 1:5 on the day of vaccination (Table 1). At time of challenge, both vaccinate groups had elevated type 1 antibody titers (≥1:126), while the NVC titers remained less than 1:5. At fetal harvest, both vaccinated and nonvaccinated animals had type 1 BVDV antibody titers (vaccinates, ≥1:244; NVCs, ≥1:435), indicating that all the animals were challenged.

### Table 1. Study 1: Geometric Mean Antibody Titers to BVDV Type 1 (BJ)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>No.</th>
<th>Vaccination (Day 0)</th>
<th>Challenge (Days 125–135)</th>
<th>C-Section (Days 200–210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>All</td>
<td>23</td>
<td>&lt;1:5</td>
<td>≥1:126</td>
<td>≥1:244</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>11</td>
<td>&lt;1:5</td>
<td>≥1:170</td>
<td>≥1:249</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>12</td>
<td>&lt;1:5</td>
<td>≥1:95</td>
<td>≥1:240</td>
</tr>
<tr>
<td>NVC</td>
<td>NA</td>
<td>10</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>≥1:435</td>
</tr>
</tbody>
</table>

### Table 2. Study 2: Geometric Mean Antibody Titers to BVDV Type 2 (PA131)

<table>
<thead>
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<th>Treatment</th>
<th>Route</th>
<th>No.</th>
<th>Vaccination (Day 0)</th>
<th>Challenge (Days 125–135)</th>
<th>C-Section (Days 200–210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>All</td>
<td>14</td>
<td>1:6</td>
<td>1:13</td>
<td>1:160</td>
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<tr>
<td></td>
<td>IM</td>
<td>6</td>
<td>1:5</td>
<td>1:8</td>
<td>1:113</td>
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<td></td>
<td>SC</td>
<td>8</td>
<td>1:7</td>
<td>1:18</td>
<td>1:207</td>
</tr>
<tr>
<td>NVC</td>
<td>NA</td>
<td>8</td>
<td>&lt;1:5</td>
<td>1:5</td>
<td>1:123</td>
</tr>
</tbody>
</table>
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Study 2

All heifers had mean baseline BVDV type 2 antibody titers of 1:7 or less on the day of vaccination (Table 2). At time of challenge, both vaccine groups had type 2 antibody titers (1:13), while the NVC titers remained 1:5. At fetal harvest, both vaccine and nonvaccine groups had type 2 BVDV antibodies (vaccine, 1:160; NVCs, 1:123), indicating that all the animals were challenged.

Virus Isolation

Study 1

Nine of 10 (90%) control heifers were pregnant at fetal harvest. All fetuses (nine of nine [100%]) were BVDV positive, indicating the challenge was effective in establishing persistent infection in calves. In the vaccinated group, 20 of 23 fetuses (87%) challenged with type 1 BVDV were negative. Ten of 11 (91%) fetuses from the IM vaccinated heifers and 10 of 12 (83%) fetuses from the SC vaccinated heifers were negative for BVDV type 1 at fetal harvest (Table 3).

Study 2

All eight (100%) fetuses from the control heifers were BVDV positive. In the vaccinated group, 13 of 14 (93%) fetuses challenged with type 2 BVDV were negative. All six (100%) fetuses from the IM vaccinated heifers and seven of eight (87%) fetuses from the SC vaccinated heifers were negative for BVDV type 2 (Table 4). The prevalence of PI fetal calves from heifers vaccinated either SC or IM differed significantly in both studies when compared with the respective control heifers in each study ($P < .01$).

<p>| TABLE 3. Protection against Type 1 Fetal Challenge |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Total No. of Animals</th>
<th>No. of Positive Animals</th>
<th>Percentage (%) PI Fetuses</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>All</td>
<td>23</td>
<td>3</td>
<td>13%</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>11</td>
<td>1</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>12</td>
<td>2</td>
<td>17%</td>
<td>83%</td>
</tr>
<tr>
<td>NVC</td>
<td>NA</td>
<td>9</td>
<td>9</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

<p>| TABLE 4. Protection against Type 2 Fetal Challenge |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Total No. of Animals</th>
<th>No. of Positive Animals</th>
<th>Percentage (%) PI Fetuses</th>
<th>Percent Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>All</td>
<td>14</td>
<td>1</td>
<td>7%</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>6</td>
<td>0</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>8</td>
<td>1</td>
<td>13%</td>
<td>87%</td>
</tr>
<tr>
<td>NVC</td>
<td>NA</td>
<td>8</td>
<td>8</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

DISCUSSION

In these studies, a monovalent type 1 NCP BVDV vaccine administered to pregnant heifers provided 87% fetal protection from challenge with a BVDV type 1a NCP strain (BJ) and 93% fetal protection from challenge with a BVDV type 2 NCP strain (PA131). In a previous study, BVDV type 1 NCP vaccines had provided protection in 25 of 27 calves.
(92%) against a type 1 NCP challenge and none of the vaccinated heifers developed viremia.13 This study is the first evidence that a BVDV type 1 NCP vaccine provided a high level of fetal protection against BVDV type 1 or type 2 challenge.

Additional fetal protection studies have been done using this same type 1 and type 2 challenge model.8,10,11 A type 1 BVDV study using a CP type 1 vaccine strain (NADL) resulted in 83% protection.10 A second study testing the efficacy of a CP type 1 (Singer strain) and CP type 2 (296 strain) vaccine resulted in 100% protection.8 Two type 2 fetal protection studies using the challenge strain PA131, with either a CP type 1 (NADL)11 or a bivalent CP type 1 and 100% fetal protection in the type 2 challenge. In another study with the same bivalent type 1 (CP) and a type 2 (CP) MLV vaccine, the level of fetal protection was 100% for the type 1 challenge and 95% in the type 2 challenge.8 However, the use of two different strains of MLV BVDV provides the opportunity for recombination and the generation of new strains.16,17

Although only NCP BVDVs are prevalent in the field and associated with field outbreaks of disease (acute outbreaks and fetal infections), only two of the more than 20 MLV BVDV vaccines available in the United States contain NCP BVDV. There has been much debate about the differences between NCP and CP BVDV vaccines. The paradigm has been that since antigenicity is the major criteria for immune response development, then a CP BVDV vaccine should be adequate for protection. However, it is clear that the pathogenesis, particularly the duration and targets of infection, are quite different between biotypes. Numerous studies have demonstrated that CP BVDV is cleared much more quickly and reaches primarily mucosal regions, whereas NCP BVDV is distributed throughout all lymphoid tissues and the virus remains in these tissues 10 to 20 days longer.18–20 NCP BVDV has a high affinity for all lymphoid tissue, increasing its opportunity for both immunosuppression and immunostimulation. Studies with a variety of NCP BVDV isolates demonstrated that the virulence of the BVDV isolate determined the severity of the interaction with the

This study is the first evidence that a BVDV type 1 NCP vaccine provided a high level of fetal protection against BVDV type 1 or type 2 challenge.
immune system, including lymphoid hyperplasia and apoptosis. Lymph nodes infected with low-virulence NCP BVDV strains contained BVDV-expressing dendritic cells and lymphoid follicles that became rapidly repopulated with T and B cells. These low-virulent NCP BVDV–infected animals could be capable of both rapid and long-term adaptive immune response.

The kinetics and character of the adaptive immune response also vary between NCP and CP BVDV. NCP viruses preferentially induce a CD4+ Th2 response, resulting in antibody being produced faster and at higher levels than CP BVDV isolates. The proliferative response is delayed with NCP BVDV compared with CP BVDV, but this depends on the virulence of the isolate. The ultimate test is the protection of the animal from infection. In a novel experiment, Lambot et al demonstrated that 100% (four of four) of animals first “vaccinated” with an NCP BVDV were protected against viremia or nasal shedding following challenge with the homologous NCP BVDV and 66% (two of three) of calves first “vaccinated” with NCP were protected from viremia against challenge with the homologous CP pair. One animal had a single positive virus isolation from a nasal swab. In contrast, only 33% of calves first “vaccinated” with a CP BVDV were protected from viremia against challenge with the same CP virus. In calves first “vaccinated” with CP BVDV, only 25% (one of four) were protected against viremia following challenge with the NCP pair.

The ability of NCP virus to spread throughout the lymphoid system has positive implications for protection against BVDV PI animals. Since the natural route of infection is through the nasal passages of the cow, followed by viremia and infection of the uterus, placenta, and, ultimately, the fetus, the ability to generate a widespread immune response is essential to prevent this infection.

**CONCLUSION**

These results support previous studies: MLV BVDV vaccines can provide fetal protection. However, the significance of the present studies is that a type 1 NCP MLV vaccine provided cross-protection after challenge with either a type 1 NCP challenge strain of BVDV (BJ) or a type 2 NCP strain (PA131). This research also supports earlier studies indicating that vaccination with an NCP BVDV strain may provide better protection than a CP strain, regardless of the challenge genotype. Using an NCP BVDV vaccine strain that has a broad level of fetal protection, like that used in the studies described here, will minimize the development of PI calves, thus aiding in the control and eradication of BVDV.

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**REFERENCES**


