Comparison of three commercial vaccines for preventing persistent infection with bovine viral diarrhea virus

Soren P. Rodning a,*, M. Shonda D. Marley b, Yijing Zhang c, Andrew B. Eason b, Callie L. Nunley a, Paul H. Walz d, Kay P. Riddell b, Patricia K. Galik c, Bruce W. Brodersen e, M. Daniel Givens b

a Department of Animal Sciences, Auburn University, Auburn, Alabama, USA
b Department of Pathobiology, Auburn University, Auburn, Alabama, USA
c Animal Health Research, Auburn University, Auburn, Alabama, USA
d Department of Clinical Sciences, Auburn University, Auburn, Alabama, USA
e Institute of Agriculture and Natural Resources, University of Nebraska Veterinary Diagnostic Center, Lincoln, Nebraska, USA

Received 25 June 2009; received in revised form 3 December 2009; accepted 4 January 2010

Abstract

Eighty crossbred beef heifers were randomly allocated to four groups to evaluate the efficacy of vaccination in preventing development of calves persistently infected with bovine viral diarrhea virus (BVDV). Group 1 (n = 11) was non-vaccinated controls, whereas three groups were vaccinated with commercially available multivalent BVDV vaccines at weaning (~7 mo of age), 28 d post-weaning, ~1 y of age, and 28 d later. Groups 2 (n = 23) and 3 (n = 23) were given a modified-live BVDV vaccine, whereas Group 4 was given an inactivated BVDV vaccine. Heifers were bred by AI and subsequently exposed to two bulls. At 61 d after AI, 70 heifers were pregnant (n = 10 for Group 1 and n = 20/group for Groups 2, 3, and 4). Three cattle persistently infected with BVDV were commingled with the pregnant heifers (in an isolated pasture) from 68 to 126 d after AI. Thereafter, viremias were detected in pregnant heifers from Groups 1, 3, and 4 (10/10, 1/20, and 10/20, respectively), but not in pregnant heifers from Group 2 (0/20). Resulting calves were assessed for persistent infection using serum PCR, ear notch antigen capture-ELISA, and immunohistochemistry. Persistently infected calves were only produced in Group 1 (10/10) and Group 4 (2/18). In conclusion, commercial vaccines provided effective fetal protection despite prolonged natural exposure to BVDV. Given that viremias were detected in 11 vaccinated heifers after BVDV exposure, and two vaccinated heifers gave birth to persistently infected calves, there is continued need for biosecurity and diagnostic surveillance, in addition to vaccination, to ensure effective BVDV control.

Keywords: Bovine viral diarrhea virus; Persistently infected; Vaccination; Fetal protection; Reproductive vaccines

Introduction

Bovine viral diarrhea virus (BVDV) is an economically important pathogen that causes gastrointestinal, respiratory, and reproductive disease in cattle. Two pathogenic biotypes of BVDV, cytopathic (CP) and noncytopathic (NCP), have been described, based on visible cytopathic effect when susceptible cell monolayers are infected [1]. Independent of biotype, viral strains are differentiated into types and subtypes (e.g., type 1a, type 1b, and type 2) based on viral RNA sequence [2,3]. Persistent infection (PI) with BVDV occurs when a bovine fetus is infected with a NCP strain.
of BVDV before 125 d of gestation [4]. The prevalence of PI animals is estimated to be < 1% [5–7]. However, PI animals develop immunotolerance to the strain or strains with which they have been infected and commonly shed large quantities of BVDV throughout life [8], thus jeopardizing efforts to eradicate or even control BVDV.

The BVDV is endemic in many parts of the world, with current control efforts based on a combination of biosecurity, diagnostic surveillance, and vaccination. Several European and Scandinavian countries currently have BVDV control programs [9–13]; some prohibit BVDV vaccination and instead require strict biosecurity and diagnostic surveillance for BVDV. However, in the USA, veterinarians and cattle producers have access to more than 150 licensed vaccines containing BVDV [14]. Therefore, the epidemiologic control afforded by vaccination needs thorough assessment to determine the best methods to control BVDV.

Although several studies have assessed fetal protection provided by vaccination against BVDV [15–25], most of these fetal challenge studies involved controlled, point-source exposure through artificial viral inoculation. Licensed vaccines often provide nearly complete protection (91–100%) of dams and developing fetuses after this artificial challenge [18,19,21,23–25]. However, exposure to PI animals may better represent viral challenge under field conditions, by increasing the duration of exposure [26]. In that regard, in a field study, two doses of a commonly used, inactivated BVDV vaccine (licensed in the USA) protected only 73% of fetuses against BVDV following a 98 d natural viral challenge involving exposure to PI cattle [27]. We inferred that prolonged BVDV field challenge may be either more severe than controlled, point-source exposure through artificial viral inoculation, or that the antigenic diversity of BVDV field strains in the USA may cause fetal infections, despite immunization with a licensed vaccine. Therefore, the goal of the present study was to assess the efficacy of vaccination with modified-live or inactivated BVDV vaccines for protection against fetal infection following viral challenge from PI animals under field conditions.

**Materials and methods**

**Cattle**

Eighty crossbred heifers born from September to November 2006 at the Upper Coastal Plain Agricultural Research Center (Alabama Agricultural Experiment Station; Winfield, AL, USA) were enrolled. Eleven of the 80 heifers exhibited an antibody titer greater than 1:4 at the initiation of the study. These animals were evenly distributed among groups due to the randomization process. Of the two control heifers that initially had antibody titers, both became seronegative by 1 y of age. Although heifers were not tested for cell-mediated immune response to BVDV, the source herd was free of BVDV, as determined by serum and white blood cell (WBC) virus isolation testing of all calves weaned in May 2006, as well as all heifer calves weaned in May 2007. Prior to beginning the study, all heifers were vaccinated against brucellosis (Brucella abortus vaccine, Strain RB-51, Professional Biological Company, Denver, CO, USA) and eight species or subtypes of Clostridia (UltraChoice® 8, Pfizer Animal Health, New York, NY, USA). Three PI animals (persistently infected with a type 1a, 1b, or 2 field strain of BVDV) were obtained from different herds. In these heifers, persistent infection with BVDV was confirmed via consistent positive results of virus isolation from serum samples obtained at least 28 d apart. Two Angus bulls, confirmed free of BVDV via serum virus isolation and semen reverse transcription nested polymerase chain reaction (RT-nPCR), were used for pasture breeding after AI.

All procedures involving cattle were approved by the Auburn University Institutional Animal Care and Use Committee (Protocol Review #2007-1172).

**Vaccination**

Vaccines were selected based on product market share for a large distributor of animal health products in the Southeastern USA. The top selling vaccines in the Spring of 2006 were two modified-live products (Bovi-Shield Gold® FP5, Pfizer Animal Health; and Pyramid® 5, Fort Dodge Animal Health, Overland Park, KS, USA) containing both CP type 1a and type 2 strains of BVDV, with label claims for fetal protection, as well as an inactivated product (Vira Shield® 6, Novartis Animal Health, Larchwood, IA, USA) containing CP type 1a, NCP type 1, and NCP type 2 strains of BVDV. Heifers were randomly allocated to four treatment groups (Table 1). Group 1 (n = 11) served as unvaccinated controls. Group 2 (Pfizer Animal Health; n = 23) and Group 3 (Fort Dodge Animal Health; n = 23) were vaccinated with modified-live products, whereas Group 4 (Novartis Animal Health; n = 23) was vaccinated with an inactivated product. Groups 2, 3, and 4 were vaccinated at weaning (~7 mo of age; May 2007), 28 d post-weaning, ~1 y of age (October 2007), and 28 d later.
Table 1
Assignment of heifers to treatment groups; those in Groups 2, 3, and 4 were vaccinated with BVDV at weaning (approximately 7 mo of age; May 2007), 28 d post-weaning, approximately 1 y of age (October 2007), and 28 d later.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unvaccinated controls</td>
</tr>
<tr>
<td>2</td>
<td>Vaccinated with a modified-live product containing both CP type 1a and type 2 strains of BVDV with a label claim for fetal protection (Bovi-Shield Gold® FPS, Pfizer Animal Health, New York, NY, USA)</td>
</tr>
<tr>
<td>3</td>
<td>Vaccinated with a modified-live product containing both CP type 1a and type 2 strains of BVDV with a label claim for fetal protection (Pyramid®, 5, Fort Dodge Animal Health, Overland Park, KS, USA)</td>
</tr>
<tr>
<td>4</td>
<td>Vaccinated with an inactivated product containing CP type 1a, NCP type 1, and NCP type 2 strains of BVDV (Vira Shield®, 6, Novartis Animal Health, Larchwood, IA, USA)</td>
</tr>
</tbody>
</table>

Housing

The 80 experimental heifers were maintained on pastures at the Upper Coastal Plain Agricultural Research Center. After each vaccination, heifers were separated into two herds for at least 28 d, based on treatment groups. Groups 1 and 4 were housed in one pasture, whereas Groups 2 and 3 were housed in a separate pasture, to prevent exposure of the control and inactivated vaccinate groups to modified-live BVDV vaccinal strains. All heifers were commingled again after at least 28 d separation post-vaccination, and were housed together during the breeding season.

Following pregnancy diagnosis, the 70 pregnant heifers were moved to an isolated area of the Upper Coastal Plain Agricultural Research Center for the BVDV challenge period (68 to 126 d after AI), during which three PI animals were housed with the pregnant heifers. All heifers were commingled again after at least 28 d separation post-vaccination, and were housed together during the breeding season.

Prior to BVDV exposure, PI animals were housed at the North Auburn BVDV Unit (Alabama Agricultural Experiment Station; Auburn, AL, USA). The PI animals were transported to the Upper Coastal Plain Agricultural Research Center for the BVDV challenge period and then returned to the North Auburn BVDV Unit.

Breeding and pregnancy determination

Estrus was synchronized in all heifers using melengestrol acetate (MGA®, Pfizer Animal Health, New York, NY, USA) and PGF2α (Lutalyse®, Pfizer Animal Health) to facilitate AI in late December 2007. Beginning in mid-November 2007, 0.5 mg/head/d melengestrol acetate was fed for 14 d. Thirty-two days after melengestrol acetate was first fed, 25 mg PGF2α (Pfizer Animal Health) was given IM. For the next 3 d, heifers were observed for estrus and bred by AI; thereafter, all remaining heifers were given 100 µg gonadorelin im (Cystorelin®, Merial, Duluth, GA, USA), and concurrently timed-inseminated. After AI of all heifers, two bulls were introduced (to breed those that were not pregnant to AI). Pregnancy status and fetal age were assessed (transrectal palpation and ultrasonography) 61 d after AI.

Viral challenge and clinical assessment

Three PI animals were commingled with the pregnant heifers 68 to 126 d after AI. General cattle health was monitored daily. Viremic status of BVDV-exposed heifers was assessed 6, 7, 8, 9, 10, 28, and 58 d after the onset of exposure. Serum neutralizing antibody titers against BVDV were assessed 28 d after the onset of exposure. Pregnancy status was reassessed via transrectal palpation 28 and 58 d after the onset of exposure (96 and 126 d post-AI).

Sample collection

Blood samples were collected from heifers at each vaccination, immediately prior to BVDV exposure, 6, 7, 8, 9, 10, 28, and 58 d after the onset of exposure, and at calving. Blood and nasal swab samples were collected from PI-exposure animals immediately prior to the BVDV challenge, as well as 10, 28, and 58 d later. Blood samples, nasal swab samples, and ear notch biopsies were collected from calves within 24 h after birth.

All serum was removed from clotted blood and processed immediately or stored at -80°C for serum neutralization assays and RT-nPCR. White blood cells,
serum, and nasal swabs were refrigerated for ≤72 h before virus isolation procedures were performed. White blood cell samples were processed as described [28] to obtain the buffy coat, except that samples were resuspended in 1 mL of Minimum Essential Medium (MEM). Ear notch skin biopsies from calves were placed in neutral-buffered 10% formalin and phosphate buffered saline prior to performing immunohistochemistry and antigen capture ELISA (HerdChek BVDV Antigen ELISA, IDEXX Laboratories, Westbrook, ME, USA), respectively.

Virus titration of serum and nasal swab samples

Virus titration of serum and nasal swab media was performed using samples from PI animals. Virus titration procedures involved multiple, serial ten-fold dilutions of 10 μL of samples diluted in 90 μL of media (performed in triplicate). The statistical method of Reed and Muench [29] was employed to determine the quantity of BVDV in nasal swab samples. An immunoperoxidase monolayer assay was performed as a labeling technique to confirm the presence of BVDV [30,31].

BVDV isolation

Virus isolation was performed on heifer serum samples collected 6, 7, 8, 9, 10, 28, and 58 d after the onset of BVDV exposure, whereas virus isolation was performed on heifer WBC samples 6, 8, 10, 28, and 58 d after the onset of exposure. Serum, buffy coat, and nasal swab samples were passaged in monolayers of Madin Darby bovine kidney (MDBK) cells in an attempt to isolate BVDV. The procedure was performed as described previously [28]. Following 4 d of incubation, the 24-well culture plates (2 cm²) underwent a single freeze-thaw cycle to release intracellular virus. Lysates from this procedure were assayed (in triplicate) by adding 10 μL of lysate sample and 90 μL MEM to a well of a 96-well culture plate, followed by the addition of 50 μL of MEM containing MDBK cells. Following 3 d of incubation, plates underwent the immunoperoxidase monolayer assay procedure, as previously described for detection of BVDV [31].

Virus neutralization

Virus neutralization assays were performed on serum samples as described previously [31], except that serum was not initially diluted. Virus neutralization was performed to investigate immune response following BVDV vaccination and natural exposure. The virus neutralization assays were performed using a genotype 1a (180) strain of BVDV found in one of the PI exposure animals. Genotype 1a was also a component of all three vaccines investigated. Serial two-fold dilutions of heat inactivated serum were made in 50 μL MEM in triplicate. Each well was then inoculated with 50 μL of MEM containing 100–300 cell culture infective dose 50 (CCID₅₀) of BVDV. After 1 h of incubation, 50 μL of MEM containing MDBK cells was added to each well. Plates were incubated for 3 d and then underwent the immunoperoxidase monolayer assay procedure, as previously described for detection of BVDV [31].

RT-nPCR and sequencing of RT-nPCR products

The RNA in serum or tissue samples from calves was extracted with QIAamp® Viral RNA preparation kit (QIAamp® Viral RNA preparation kit, Qiagen, Valencia, CA, USA). An aliquot (140 μL) was extracted to yield 60 μL of sample, which was assayed using RT-nPCR, as described previously [32]. The outer primers, BVD 100 (5′-GGCTAGCCATGCCCTTAG-3′) and HCV 368 (5′-CCATGTGCCATGTACAG-3′) amplified a 290 base pair sequence of the 5′ nontranslated region of the viral genome, whereas the inner primers, BVD 180 (5′-CCTGAGTACAGGGDAGTCGTCA-3′) and HCV 368, amplified a 213 base pair sequence within the first amplicon. When a sample was positive for BVDV, it was amplified in triplicate. The resulting RT-nPCR products were purified using a silica gel-based membrane kit (QIAquick® PCR Purification Kit, Qiagen, Valencia, CA, USA) and sequenced with automated dye terminator nucleotide sequencing, using both the 5′ and 3′ primers (BVD 180 and HCV 368, respectively). Consensus sequences were determined using Align X computer software (Vector NTI Advance 10, Invitrogen, Carlsbad, CA, USA), and compared to sequences from the PI animals used for viral exposure.

Immunohistochemistry

Ear notch skin biopsies were placed in neutral-buffered 10% formalin for transport to the University of Nebraska Veterinary Diagnostic Center (Lincoln, Nebraska, USA) for immunohistochemical detection of viral antigen [31].

Antigen capture ELISA

Ear notch skin biopsies were transported (in phosphate buffered saline) to the Thompson-Bishop-
Sparks Veterinary Diagnostic Laboratory (Alabama Department of Agriculture and Industries, Auburn, AL, USA) for detection of viral antigen by a commercially available antigen capture ELISA (IDEXX Laboratories).

Data analysis

Data were analyzed using JMP software (JMP, SAS Institute Inc., Cary, NC, USA). Geometric means of the reciprocal of the antibody titers were analyzed using repeated measures ANOVA, and a significant difference among treatment groups was noted over time. Therefore, geometric means of the reciprocal of the antibody titers on evaluated days were compared using an ANOVA F-test. Detection of viremia, pregnancy loss, and production of persistently infected offspring were analyzed by comparing each treatment group to unvaccinated controls (Group 1), using the Fisher exact test. Furthermore, differences in these three categorical outcomes were analyzed by combining results of heifers receiving modified-live vaccines (Groups 2 and 3) and comparing (Fisher exact test) those outcomes to those in heifers receiving an inactivated vaccine (Group 4). As all aborted fetuses were lost to determination of the status of persistent infection with BVDV, aborting heifers were excluded from analysis of the production of persistently infected offspring. For all analyses, \( P \leq 0.05 \) was considered significant.

Results

Seventy heifers became pregnant (\( n = 10 \) for Group 1; \( n = 20 \) for Groups 2, 3, and 4). Based on fetal measurements done with ultrasonography, pregnancy was achieved by AI and natural service in 49 and 21 heifers, respectively. Upon introduction of PI exposure animals, fetal ages ranged from 30–68 d. No abortions were apparent based on transrectal palpation 28 and 58 d after the onset of BVDV exposure (96 and 126 d after AI). However, five heifers aborted after the last pregnancy reassessment (\( n = 1 \) for Group 2; \( n = 2 \) for Groups 3 and 4). Unfortunately, none of the fetuses were recovered. The gestational age for 4 of the 5 aborted fetuses was 68 d when PI exposure animals were introduced, and 32 d for the remaining aborted fetus. The two heifers that aborted in Group 4 were viremic between 6 and 10 d after the onset of BVDV exposure (type 1a and 2, respectively), but no viremias were detected in heifers that aborted in Groups 2 and 3. The abortion rate of vaccinated groups did not differ significantly from the abortion rate of the control group, nor did the abortion rate of heifers receiving modified-live vaccines significantly differ from the abortion rate of heifers receiving an inactivated vaccine.

Geometric mean antibody titers to BVDV type 1a were summarized (Fig. 1). Immediately prior to introduction of the PI animals, Group 3 had the highest concentration of BVDV-neutralizing antibodies, followed by Groups 4 and 2, respectively. Group 1 exhibited no BVDV-neutralizing antibodies prior to introduction of the PI animals. There were significant differences among the four treatment groups in geometric mean antibody titers to BVDV type 1a.

Heifer serum and WBC virus isolation results were summarized (Tables 2 and 3, and Fig. 2). Virus isolation was performed on serum samples collected 6, 7, 8, 9, 10, 28, and 58 d after the onset of BVDV exposure, whereas virus isolation was performed on heifer WBC samples 6, 8, 10, 28, and 58 d after the onset of exposure. Viremias were detected in heifers from Group 1 (10/10), Group 3 (1/20), and Group 4 (10/20), but no viremias were detected in Group 2 (0/20). Between 6 to 10 d after the onset of exposure, viremias were detected only in heifers from Group 1 (10/10; four type 1a, two type 1b, and four type 2) and Group 4 (9/20; three type 1a, two type 1b, and four type 2). Additional heifers were viremic in Group 3 (type 2) and Group 4 (type 1b) 28 d after the onset of exposure. No heifers were viremic 58 d after the onset of exposure (day of PI removal). Virus isolation from WBC was more successful than virus isolation from serum (Table 2). Of the 21 heifers that were positive via virus isolation from WBC, only eight were positive via virus isolation from serum.

Bovine viral diarrhea virus was isolated from all PI exposure animal serum and nasal swab samples obtained prior to and during the BVDV-exposure period. Serum titers ranged from \( 3.5 \times 10^3 \) to \( 5.2 \times 10^4 \) CCID50/mL and nasal titers ranged from \( 3.5 \times 10^4 \) to \( 2 \times 10^7 \) CCID50/mL throughout the exposure period (Table 4). One PI animal used for BVDV exposure developed coronary band ulcerations with mild lameness, but recovered with minor medical treatment and was returned to the North Auburn BVDV Unit with the other PI exposure animals at the end of the BVDV challenge period.

Calves were born over a 40 d interval in September and October 2008. The number of PI calves produced was summarized (Fig. 3) based on results of calf ear notch antigen capture ELISA (IDEXX Laboratories), immunohistochemistry, and serum RT-nPCR (Table 2). Persistently infected calves (\( n = 12 \)) were only produced in Group 1 (10/10) and Group 4 (2/18), with no PI calves produced in Group 2 (0/19) or Group 3 (0/18).
The production of persistently infected offspring from each vaccinated group differed from the production of persistently infected offspring from unvaccinated controls (Group 1; \( p < 0.0001 \)). The difference in production of persistently infected offspring between heifers receiving modified-live vaccine (Groups 2 and 3) and heifers receiving inactivated vaccine (Group 4) did not reach significance (\( P = 0.103 \)).

All PI calves were positive within 24 h after birth, on the basis of serum RT-nPCR and ear notch immunohistochemistry, whereas 11 of 12 PI calves were positive at birth via ear notch antigen capture ELISA (IDEXX Laboratories). One PI calf from Group 1 was negative at birth via ear notch antigen capture ELISA (IDEXX Laboratories), but was positive via RT-nPCR and immunohistochemistry. However, this calf was subsequently positive via serum RT-nPCR, ear notch immunohistochemistry, and antigen capture ELISA (IDEXX Laboratories) at 25 d of age.

With respect to neonatal calf nasal swab samples, only 4 of 12 nasal swabs from PI calves were positive via virus isolation. An attempt was made to collect calf nasal swab samples prior to colostrum absorption, but this was only possible for 5 of 12 PI calves. Of the 5 nasal swabs collected prior to colostrum absorption, 4 were positive via virus isolation.

Table 2
Results of virus isolation testing for BVDV in the serum and white blood cells of heifers following exposure to animals persistently infected with BVDV, and results of antigen capture ELISA, immunohistochemistry and RT-nPCR assays to detect BVDV in resulting offspring.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Heifers</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus isolation from white blood cells (positive/total samples)</td>
<td>Virus isolation from serum (positive/total samples)</td>
</tr>
<tr>
<td>1</td>
<td>10/10</td>
<td>8/10</td>
</tr>
<tr>
<td>2</td>
<td>0/20*</td>
<td>0/20*</td>
</tr>
<tr>
<td>3</td>
<td>1/20*</td>
<td>0/20*</td>
</tr>
<tr>
<td>4</td>
<td>10/20*</td>
<td>0/20*</td>
</tr>
</tbody>
</table>

* Results differed significantly from unvaccinated controls (Group 1).

* Results from heifers receiving inactivated vaccine (Group 4) differed significantly from heifers receiving modified-live vaccine (Groups 2 and 3 combined).
The association between heifer viremia and development of PI calves and their respective genotype was examined. Eight out of ten PI calves produced in Group 1 were infected with the same genotype as their respective dam’s viremia (four type 1a, two type 1b, and two type 2). One of the remaining PI calves in Group 1 was infected with two genotypes (type 1b and 2), one of which was the same genotype as its dam’s detected viremia (type 2). The final PI calf in Group 1 was infected with BVDV type 1a, whereas the viremia detected in its dam was type 2. Of the 2 PI calves produced in Group 4, one was infected with the same genotype as its dam’s viremia (type 1b). No viremia was detected in the dam of the remaining PI calf (type 2) from Group 4. In addition, one viremic heifer in Group 3 and nine viremic heifers in Group 4 did not give birth to PI calves.

Discussion

The goal of this study was to investigate the efficacy of several commonly used BVDV vaccines, licensed for sale in the USA, including both modified-live (Pfizer Animal Health and Fort Dodge Animal Health) and inactivated (Novartis Animal Health) vaccines, for protection against fetal infection, despite prolonged viral challenge from PI animals under field conditions. The challenge-exposure method implemented in this study was effective and rigorous, as determined by evaluation of the following end points. All control heifers were seronegative for antibodies to BVDV type 1a prior to BVDV challenge exposure. After challenge exposure, all control heifers subsequently became viremic, developed BVDV-neutralizing antibodies,

Table 3

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>BVDV genotype 1 (n = 10)</th>
<th>2 (n = 20)</th>
<th>3 (n = 20)</th>
<th>4 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1a</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Type 1b</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Type 2</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>BVDV genotype</th>
<th>Sample</th>
<th>Day PI animals were introduced</th>
<th>10 d post-introduction</th>
<th>28 d post-introduction</th>
<th>58 d post- introduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1a</td>
<td>Serum</td>
<td>$3.5 \times 10^4$</td>
<td>$2.0 \times 10^5$</td>
<td>$3.5 \times 10^4$</td>
<td>$2.0 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>$6.2 \times 10^5$</td>
<td>$2.0 \times 10^5$</td>
<td>$1.1 \times 10^7$</td>
<td>$3.5 \times 10^4$</td>
</tr>
<tr>
<td>Type 1b</td>
<td>Serum</td>
<td>$3.5 \times 10^4$</td>
<td>$5.2 \times 10^5$</td>
<td>$3.5 \times 10^4$</td>
<td>$2.0 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>$3.5 \times 10^5$</td>
<td>$3.5 \times 10^5$</td>
<td>$2.0 \times 10^7$</td>
<td>$3.5 \times 10^5$</td>
</tr>
<tr>
<td>Type 2</td>
<td>Serum</td>
<td>$3.5 \times 10^4$</td>
<td>$3.5 \times 10^4$</td>
<td>$6.2 \times 10^3$</td>
<td>$3.5 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>$3.5 \times 10^5$</td>
<td>$3.5 \times 10^5$</td>
<td>$3.5 \times 10^5$</td>
<td>$6.2 \times 10^4$</td>
</tr>
</tbody>
</table>
and gave birth to PI calves. In addition, viremias were detected in 11 of the vaccinated heifers following BVDV exposure, and two vaccinated heifers gave birth to PI calves. Geometric mean antibody titers for vaccinated groups also increased following the BVDV challenge exposure. The fact that two heifers were viremic 28 d after the onset of exposure indicated the continued presence of circulating BVDV. Based on these data, our interpretation was that all treatment groups were exposed to BVDV over a prolonged, rigorous field challenge.

Vaccination with four doses of a commercially available multivalent vaccine containing modified-live BVDV (Pfizer Animal Health and Fort Dodge Animal Health) provided greater protection against viremia and the production of PI calves than vaccination with four doses of a commercially available multivalent vaccine containing inactivated BVDV (Novartis Animal Health). In this study, PI animals were introduced to pregnant heifers during a time when fetuses are susceptible to developing persistent infections. Four vaccinations with modified-live BVDV between weaning and breeding provided 100% protection against the development of PI calves after this worst-case scenario. Four vaccinations with inactivated BVDV between weaning and breeding provided 89% protection against the development of PI calves—notably better than no vaccination. However, viremias were detected in one modified-live vaccinate and 10 inactivated vaccinates after BVDV exposure, and two inactivated vaccinates gave birth to PI calves.

While the administration of four doses of vaccine in the experimental design of this research is subject to criticism for exceeding common practice in the field, previous research by Grooms et al. [27] had demonstrated that two doses of an inactivated vaccine containing BVDV given to heifers 69 d before exposure for a duration of 98 d to cattle PI with diverse viral subtypes resulted in 4 of 15 (27%) offspring being PI. Prior research by Ellsworth et al. [33] demonstrated that a dose of modified-live vaccine containing BVDV given 490 d before exposure for a duration of 45 d to cattle PI with type 2a—similar to the vaccine strain—prevented congenital infections in 95% of offspring. Additionally, Patel et al. [17] demonstrated that two doses of an inactivated vaccine containing BVDV given to heifers 187 d before exposure for a duration of 14 d to cattle PI with type 1a—similar to the vaccine strain—prevented persistent infection in 11 of 11 offspring. Thus, the current study was apparently the first to demonstrate 100% prevention of persistent infection when viral challenge included exposure to cattle PI with viral subtypes not found within the vaccine. The design of the current study was based on what many producers consider to be an unacceptable rate of PI offspring (27%) following administration of two doses of inactivated product and reflected an attempt to identify vaccination protocols that will consistently prevent persistent infection of offspring in the face of a stringent viral challenge under field conditions.

That most vaccines lack BVDV type 1b strains has been suggested as a reason that type 1b predominates in the cattle population and causes the majority of detected PI animals [34,35]. In this study, the two modified-live vaccines contained both CP type 1a and type 2 strains of BVDV (Pfizer Animal Health and Fort Dodge Animal Health), whereas the inactivated vaccine contained CP type 1a, NCP type 1, and NCP type 2 strains of BVDV (Novartis Animal Health). Despite the lack of vaccine BVDV type 1b strains, only one type 1b PI calf was produced in the vaccinated groups, indicating effective immunity against NCP type 1b following four vaccinations with NCP type 1 and/or CP type 1a BVDV. We inferred that the lack of BVDV type 1b vaccinal strains may not be the underlying cause of the predominance of type 1b in the cattle population or in the majority of diagnosed PI calves. Perhaps animals persistently infected with type 1b survive longer or shed BVDV more efficiently, resulting in the skewed distribution of BVDV subgenotypes.

Virus isolation was affected by treatment group. Virus was isolated from more heifers in Groups 1 and 4 during the first 28 d after the onset of BVDV exposure compared to Groups 2 and 3, indicating significantly better protection against viremia following vaccination with modified-live BVDV vaccines (Table 2). Since viremia likely precedes fetal infection, prevention of viremia via vaccination is desired. However, not all viremias resulted in the development of PI calves. Of the 21 heifers in which viremia was detected, only 11 gave birth to PI calves. All 10 viremic heifers in Group 1 gave birth to PI calves, whereas only one out of 10 viremic heifers in Group 4 gave birth to a PI calf. Therefore, the inactivated vaccine used in this study provided fetal protection in the face of viremia in 9 of 10 instances.

Virus isolation from WBC detected more viremias than virus isolation from serum. Of the 21 heifers that were positive via virus isolation from WBC, only eight were positive via virus isolation from serum. However, all eight heifers that were positive via virus isolation from serum gave birth to PI calves, whereas PI calves were produced from only 11 of 21 heifers that were positive via virus isolation from WBC. In this study, isolating BVDV from pregnant heifer serum between 74 and 96 d of
pregnancy had a positive predictive value of 100% for the development of PI calves, compared to a positive predictive value of only 52% for BVDV isolation from heifer WBC. The detection of BVDV in WBC of vaccinates without resulting development of PI offspring was consistent with results of Patel et al. [17]. As expected, the majority of PI calves were infected with the same BVDV genotype as their respective dam’s detected viremia. Based on these results, our interpretation was that virus isolation from WBC was a better diagnostic screening test for pregnant cattle recently exposed to BVDV. However, virus isolation from serum was a better predictor of fetal infection and likely indicated a more severe infection for the dam as well.

Not all viremias were detected; one heifer in Group 4 in which no viremia was detected also gave birth to a PI calf. Viremias were likely missed due to the decreased sensitivity of our sampling regimen. For logistical reasons, virus isolation from WBC was only attempted 6, 8, 10, 28, and 58 d after the onset of BVDV exposure, as opposed to virus isolation from serum 6, 7, 8, 9, 10, 28, and 58 d after the onset of exposure. Given the decreased sensitivity of virus isolation from serum, it is quite possible due to the short duration of BVDV viremias (eight heifers were positive via virus isolation from WBC on only 1 d) that additional viremic heifers were missed 7, 9, or greater than 10 d after the onset of exposure. Although future diagnostic regimens could include collection of addition samples for virus isolation from WBC, such a rigorous testing regimen was not considered necessary, since the primary objective was to evaluate the development of PI calves.

Neonatal nasal swab virus isolation yielded interesting results. Despite attempts to collect calf nasal swab samples prior to colostrum absorption, this was only possible for 5 of 12 PI calves. As a result, all seven nasal swab samples collected after colostrum absorption were negative via virus isolation, most likely due to neutralization of virus by passively-derived BVDV-neutralizing antibodies. However, of the five nasal swab samples collected prior to colostrum absorption, four were positive via virus isolation, indicating that nasal swab virus isolation might be a reliable diagnostic methodology for determining PI status of neonatal animals prior to colostrum absorption. Perhaps more importantly, we inferred that there was rapid delivery of passively-derived BVDV antibodies to the nasal mucosa, and limited nasal shedding of BVDV during the early life of a PI calf.

In summary, vaccination with four doses of commercially available multivalent vaccines containing modified-live or inactivated BVDV prior to breeding significantly reduced the risk of BVDV fetal infections in pregnant heifers exposed to BVDV via prolonged exposure to PI cattle. However, despite four vaccinations, viremias were detected in 11 vaccinated heifers after BVDV exposure and two heifers given vaccines containing inactivated BVDV subsequently gave birth to PI calves, thereby emphasizing the need for biosecurity and diagnostic surveillance, in addition to vaccination, to ensure effective BVDV control.

Acknowledgments

This study was supported by a grant from the Alabama Agricultural Experiment Station.

References


