Efficacy of four commercially available multivalent modified-live virus vaccines against clinical disease, viremia, and viral shedding in early-weaned beef calves exposed simultaneously to cattle persistently infected with bovine viral diarrhea virus and cattle acutely infected with bovine herpesvirus 1

Manuel F. Chamorro DVM, PhD
Paul H. Walz DVM, PhD
Thomas Passler DVM, PhD
Roberto Palomares DVM, PhD
Benjamin W. Newcomer DVM, PhD
Kay P. Riddell DVM, MS
Julie Gard DVM, PhD
Yijing Zhang BS
Patricia Galik MS

OBJECTIVE
To evaluate the efficacy of 4 commercially available multivalent modified-live virus vaccines against clinical disease, viremia, and viral shedding caused by bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV1) in early-weaned beef calves.

ANIMALS
54 early-weaned beef steers (median age, 95 days).

PROCEDURES
Calves were randomly assigned to 1 of 5 groups and administered PBSS (group A [control]; n = 11) or 1 of 4 commercially available modified-live virus vaccines that contained antigens against BHV1, BVDV types 1 (BVDV1) and 2 (BVDV2), parainfluenza type 3 virus, and bovine respiratory syncytial virus (groups B [11], C [10], D [11], and E [11]). Forty-five days after vaccination, calves were exposed simultaneously to 6 cattle persistently infected with BVDV and 8 calves acutely infected with BHV1 for 28 days (challenge exposure). For each calf, serum antibody titers against BVDV and BHV1 were determined before vaccination and before and after challenge exposure. Virus isolation was performed on nasal secretions, serum, and WBCs at predetermined times during the 28-day challenge exposure.

RESULTS
None of the calves developed severe clinical disease or died. Mean serum anti-BHV1 antibody titers did not differ significantly among the treatment groups at any time and gradually declined during the study. Mean serum anti-BVDV antibody titers appeared to be negatively associated with the incidence of viremia and BVDV shedding. The unvaccinated group (A) had the lowest mean serum anti-BVDV antibody titers. The mean serum anti-BVDV antibody titers for group D were generally lower than those for groups B, C, and E.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated differences in vaccine efficacy for the prevention of BVDV viremia and shedding in early-weaned beef calves. (Am J Vet Res 2016;77:88–97)
antibodies and then exposed to BVDV and BHV1. Protection against clinical disease is positively associated with the activation of naïve B and T lymphocytes that induce virus-specific humoral and anamnestic cell-mediated immune responses.\textsuperscript{11-15}

Aside from protecting calves against clinical disease, effective vaccines reduce viremia and viral shedding following exposure to BVDV and BHV1.\textsuperscript{9,16} Results of previous studies\textsuperscript{17-19} indicate that administration of 1 dose of a multivalent MLV vaccine containing antigens against BVDV and BHV1 to calves with maternally derived antibodies protects those calves against clinical disease when they are subsequently exposed to those viruses. However, to our knowledge, the efficacy of multivalent MLV vaccines for prevention of viremia and viral shedding following exposure of calves to BVDV and BHV1 has not been evaluated. The primary objective of the study reported here was to evaluate the efficacy of 4 commercially available, multivalent MLV vaccines against clinical disease, viremia, and viral shedding in early-weaned beef calves that were subsequently exposed simultaneously to cattle PI with BVDV and cattle acutely infected with BHV1. A secondary objective was to investigate the ability of the 4 vaccines to induce antibody responses against BVDV and BHV1 in calves with various serum concentrations of maternally derived antibodies.

Materials and Methods

Animals

Fifty-four clinically normal crossbred beef steer calves that were born into the 200-cow beef cattle unit at the Upper Coastal Plain Agricultural Research Center in Winfield, Ala, that is operated by Auburn University were enrolled in the study. Only male calves born between September 1 and October 1, 2013, to cows that had received 1 dose of a multivalent MLV vaccine,\textsuperscript{4} which contained antigens against BHV1, BVDV1, BVDV2, P13V, and BRSV, prior to breeding were eligible for study enrollment. Each calf was individually identified by a tattoo and plastic tag that were applied to an ear immediately after birth. All calves remained with their dams and consumed colostrum and milk under natural conditions while housed on pasture until weaned.

Experimental design

All study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University. The study was designed as a randomized controlled trial that consisted of 5 treatment groups (an unvaccinated control group [group A], and a group [groups B through E] for each of 4 multivalent MLV vaccines). Sample size was determined with the power calculation procedure of a commercially available statistical software program\textsuperscript{5} on the basis of the following equation:

\[
n = \left( \frac{1 + 1}{r_1 \times r_2} \right) \sigma^2 \left( \frac{z_{1-\alpha/2} + z_{1-\beta}}{\delta} \right)^2
\]

where \( n \) is the number of animals required, \( r_1 \) is the proportion of subjects in group 1, \( r_2 \) is the proportion of subjects in group 2, \( \sigma^2 \) is the population variance, \( z_{1-\alpha/2} \) is the value of the standard normal distribution corresponding to \( 1 - \alpha/2 \) (ie, 1.96, when \( \alpha = 0.05 \)), \( z_{1-\beta} \) is the value of the standard normal distribution corresponding to \( 1 - \beta \) (ie, 1.645, when power is estimated to be 95% [\( \beta = 0.05 \)]), and \( \delta \) is the expected difference between the mean log\textsubscript{2} anti-BVDV1 antibody titer between the 2 groups. The expected mean log\textsubscript{2} antibody titer against BVDV1 was 4.0 for unvaccinated calves and 8.0 for vaccinated calves with an estimated SD of 2.5 for both unvaccinated and vaccinated calves. The type I error rate was 0.05, the desired power was 95%, and a 2-sided, 2-sample test was used.

Introduction of calves to cattle PI with BVDV (PI cattle) and cattle acutely infected with BHV1 was designated as study day 0. A blood sample (10 mL) was obtained by jugular venipuncture from each calf for determination of SNA titers against BVDV1, BVDV2, and BHV1 on day –75 (median calf age, 40 days). On day –45, all calves (median age, 95 days; range, 60 to 128 days) were weaned and randomly assigned to receive either PBSS (control) or 1 of 4 commercially available multivalent MLV vaccines. The initial SNA titers against BVDV1a determined for blood samples collected on day –75 varied substantially among calves; therefore, the calves were stratified into 3 strata on the basis of their initial anti-BVDV1a antibody titers, which were assumed to be maternally derived. Within each strata, calves were randomly assigned to 1 of 5 treatment groups by use of a random number generator.\textsuperscript{8} This ensured that each treatment group was composed of a similar number of calves with the various concentrations of maternally derived anti-BVDV1a antibodies.

Calves were vaccinated once with PBSS or the assigned vaccine on day –45. To ensure that the personnel administering the vaccines remained unaware of (ie, were blinded to) the treatment allocation for each group, they were provided with 5 plastic bags, each of which was labeled with the group identification (A, B, C, D, or E) and held 11 2-mL syringes that contained the designated treatment. Following vaccination, each group of calves was isolated in a separate pasture for 28 days to prevent transmission of vaccine viral strains among groups. Farm personnel who were blinded to the treatment allocation of each group observed the calves daily for evidence of adverse vaccine reactions or other health problems.

On day –17 (28 days after vaccination), all calves were commingled in 1 pasture. On day –1 (44 days after vaccination), calves were transported 507 km to the North Auburn BVDV Isolation Unit located in Auburn, Ala. Calves were provided access to fresh water and hay at arrival. On day 0, 6 PI cattle and 8 calves acutely infected with BHV1 were introduced into the pen where the calves were commingled. The calves re...
maintained exposed to and shared water and feed sources with the PI cattle and BHV1-infected calves for 28 days (challenge exposure).

**Vaccines**

Calves in group A were administered PBSS (2 mL, SC; control; n = 11). Calves in groups B (n = 11), C (10), D (11), and E (11) were administered vaccine B,d C,e D,d or E' (2 mL, SC), respectively. All vaccines were licensed and approved for sale commercially by the USDA and administered in accordance with the label directions. Vaccines B, C, and D were MLV vaccines that contained antigens against BHV1, BVDV1a, BVDV2, PI3V, and BRSV. Vaccine E was an MLV vaccine that contained antigens against BHV1, BVDV1a, BVDV1b, BVDV2, PI3V, and BRSV.

**Cattle PI with BVDV**

The 6 PI cattle used for the BVDV challenge exposure consisted of 2 cattle PI with BVDV1a, 2 cattle PI with BVDV1b, and 2 cattle PI with BVDV2. A blood sample (10 mL) to obtain serum and a nasal swab specimen were obtained from each PI animal for virus isolation and titration (10-fold dilution) to confirm that it was shedding BVDV on days 0 (immediately prior to challenge exposure) and 28. The PI cattle were vaccinated with a multivalent MLV vaccine8 (2 mL, intranasally) that contained antigens against BHV1, PI3V, and BRSV on day –8.

**Calves acutely infected with BHV1**

Eight 4-month-old steers that were seronegative for antibodies against BHV1 were inoculated with 3 × 10^7 CCID^50 of BHV1 (Colorado strain).h IV. Four calves were inoculated on day –7, and the other 4 were inoculated on day –5 to ensure that they were in the acute stage of BHV1 infection when introduced to the study calves. From each BHV1-inoculated calf, a blood sample (10 mL) and nasal swab specimen were obtained for virus isolation on days 0, 3, 7, and 14.

**Sample and data collection**

Calves were visually evaluated on days 0, 3, 6, 7, 8, 9, 10, 14, 21, and 28 by a veterinarian (KPR) who was blinded to the treatment group assignment of the individual calves and was responsible only for assessing calves for signs of abnormal respiration, depression, and diarrhea. On each day, each calf was assigned 3 scores on a scale of 0 to 3 as described20 to characterize whether it had abnormal respiration, depression, or diarrhea. Briefly, calves without clinical signs of disease were assigned a score of 0, whereas those with severe clinical signs of disease were assigned a score of 3. Respiration was considered abnormal (score > 0) if a calf had a nasal discharge and the nasal discharge was classified as serous (1), mucoid (2), or mucopurulent (3). Depression was classified as none (0), mild (1), moderate (2), or severe (3). Feces were subjectively evaluated for consistency and were classified as normal (0), pasty (1), runny (2), or severe diarrhea (3). Rectal temperature, deep nasal swab specimens, and blood samples collected by jugular venipuncture into a serum separator tube (10 mL; serum) and a tube containing EDTA (10 mL; whole blood) were obtained from each calf following visual evaluation on days 0, 3, 6, 7, 8, 9, 10, 14, 21, and 28. Investigators (MFC, PHW, and TP) who were not blinded to the treatment allocation of individual calves collected the samples; however, the samples were labeled such that the personnel processing the samples in the laboratory remained blinded to the treatment allocation. Laboratory personnel who processed and analyzed the samples did not participate in the assignment of calves to the various treatment groups or sample collection.

For each calf, body weight was determined on days –45 (weaning and vaccination), 0 (initiation of challenge exposure), 14, 28 (end of challenge exposure), and 56. A portable electric livestock scale1 was used to weigh individual calves, and the scale was calibrated before and after all weights were obtained on each day.

**Virus titration**

Virus titration was performed as described20 on serum samples and nasal swab specimens obtained from the 6 PI cattle on days 0 and 28 and the 8 BHV1-infected calves on days 0, 3, 7, and 14. Briefly, serial 10-fold dilutions (1:10 to 1:10,000,000) of serum and nasal swab specimen fluid samples were created and evaluated in triplicate. The method of Reed and Muench21 was used to determine the quantity of BVDV or BHV1 in each sample. For each dilution of serum or nasal secretions, 10 µL was added to each of 3 wells of a 96-well plate, then 90 µL of MEM was added to each well. Each well was subsequently seeded with approximately 2.5 X 10^3 MDBK cells in 50 µL of MEM. To determine the BVDV titer, plates were incubated for 3 days at 37°C in humidified air that contained 5% CO_2 and air. An immunoperoxidase monolayer assay was used to identify BVDV in the cultured MDBK cells. To determine the BHV1 titer, plates were incubated for 5 days at 38.5°C in humidified air that contained 5% CO_2. The wells were examined daily for the presence of the characteristic cytopathic effect associated with BHV1.
PCR assays were developed as described.23 Those protocols to detect either DNA from nasal swab specimens. Protocols to detect that contained BVDV were identified by the use of an immunoperoxidase monolayer assay as described.22 For that contained nasal swab fluid samples developed cytopathic effects; therefore, a qPCR assay was performed on all nasal swab fluid samples to identify BVDV RNA and BHV1 DNA. Real-time qPCR assays During virus detection, many of the cells in wells that contained nasal swab fluid samples developed cytopathic effects; therefore, a qPCR assay was performed on all nasal swab fluid samples to identify BVDV RNA and BHV1 DNA. A membrane kit was used in accordance with the manufacturer’s instructions to extract viral RNA or DNA from nasal swab specimens. Protocols to detect BVDV and BHV1 by use of SYBR green–based real-time PCR assays were developed as described.23 Those protocols were used to establish the optimal PCR conditions necessary to detect the lowest concentration of the standard template required for amplification of the correct gene fragment for the virus (BVDV or BHV1) being evaluated. The forward and reverse primers (5′ to 3′) used for BVDV were TAGCCATGCCCTTAGGAGC and GACGACTACCTGTACTCGG, respectively, and those for BHV1 were GTAAGGGTATATTATTGATTGC and GACGACTACCCTGTACTCAGG, respectively, and those for BHV1 were GTAAGGGTATATTATTGATTGC and GACGACTACCCTGTACTCAGG, respectively. For the BVDV qPCR assay, the reaction mixture in each tube consisted of 5 µL of RNA template, 10 µL of an SYBR green master mix, 0.25 µL of reverse transcriptase, 0.3 µM (0.3 µL) of both the forward and reverse primers, and sufficient sterile PCR-grade water to bring the total reaction volume to 20 µL. The BVDV PCR protocol consisted of 10 minutes at 50°C for 1 cycle and 1 minute at 95°C for 1 cycle, followed by 10 seconds at 95°C and 30 seconds at 56.5°C for 39 cycles. A positive control was maintained with the BVDV standard template, and a negative control was maintained with PCR-grade water instead of the RNA template. An additional control without reverse transcriptase was also maintained for the BVDV qPCR assay. The expected qPCR BVDV product consisted of 128 bp. For the BHV1 qPCR assay, the reaction mixture in each tube consisted of 5 µL of DNA template, 10 µL of an SYBR green super mix, 0.5 µM (0.5 µL) of both the forward and reverse primers, and sufficient sterile PCR-grade water to bring the total reaction volume to 20 µL. The BHV1 PCR protocol consisted of 3 minutes at 98°C for 1 cycle, followed by 15 seconds at 95°C and 3 seconds at 60°C for 35 cycles. A positive control was maintained with the BHV1 standard template, and a negative control was maintained with PCR-grade water instead of the RNA template. The expected qPCR BHV1 product consisted of 213 bp. A real-time PCR detection system was used for all reactions.

Determination of BVDV genotype For WBC samples from which BVDV was isolated, a 2-round, rapid-cycle RT-nPCR assay was performed to determine the viral genotype as described.20 Positive and negative controls were included in the RNA extraction step. For each sample, all steps of the RT-nPCR assay were performed in 1 tube. During the first round, the outer primers BVD 100 (5′-GGCTAGCCCATGCCCCTTAG-3′) and HCV 368 (5′-CCATGTGCCATGTCAG-3′) were used to amplify a 290-bp sequence of the 5′ untranslated region of the viral genome. During the second round, the inner primers BVD 180 (5′-CCTGAGTACAGGDA-GTCGTC-3′) and HCV 368 were used to amplify a 213-bp sequence within the first amplicon. Following completion of the second round, 10 µL of the RT-nPCR products was separated by 1.5% agarose gel electrophoresis. The resulting gels were stained with a nucleic acid stain and transilluminated with UV light to visually identify the RT-nPCR products. For samples confirmed to contain BVDV, viral RNA was amplified in triplicate. The resulting RT-nPCR products were then purified by use of a silica gel–based membrane kit and sequenced with automated dye terminator nucleotide sequencing by use of both the 5′ (BVD 180) and 3′ (HCV 368) primers. Consensus sequences were determined with computer software and compared with the BVDV sequences obtained from the 6 PI cattle used for the BVDV challenge exposure to determine the genotype of the infecting virus.

Serum virus neutralization assays Standard virus neutralization microtiter assays were performed as described22 to detect antibodies against BVDV and BHV1 in serum harvested from blood samples obtained on days –75, –45, 0, and 28. The reference strains used in the assays were the cytopathic NADL strain for BVDV1a, cytopathic TGAC strain for BVDV1b, cytopathic 125C strain for BVDV2, and Colorado strain for BHV1. Serum samples were heat inactivated at 56°C for 30 minutes. For each sample, serial 2-fold dilutions were prepared with 50 µL of MEM in triplicate in wells of a 96-well plate. To each well, 50 µL of MEM containing 100 to 500 CCID50 of the designated virus reference strain was added. The plates were incubated for 1 hour at 38.5°C in humidified air that contained 5% CO2. Then, 2.5 × 103 MDBK cells in 50 µL of MEM were added to each well. For the BVDV neutralization assays, plates were incubated for 72 hours and each well was visually evaluated for cytopathic effects daily. For the BHV1 neutralization assay, after each well was inoculated with the MDBK cells, plates were incubated for 96 hours at 38.5°C in humidified air that contained 5% CO2 and each well was visually evaluated for cytopathic effects daily. The SNA titer for each sample was calculated as the serum dilution at which half of the wells had evidence of cytopathic effects.
Hematologic analysis

A CBC was performed on whole blood samples obtained on days 0, 8, and 14. For each sample, the WBC and platelet counts were determined by use of an automatic cell counter.

Statistical analysis

Outcomes of interest for the study were the overall morbidity (as determined by the clinical evaluation scores) and mortality rates, rates of BVDV and BHV1 viremia and viral shedding, SNA titers against BVDV and BHV1 (used as surrogate measures of protection against clinical disease), WBC and platelet counts, and weight gain. The distribution of data for continuous variables (SNA titers against BVDV and BHV1, rectal temperature, WBC and platelet counts, and body weight) was assessed for normality by means of the Kolgomorov-Sminov and Shapiro-Wilk tests. The geometric mean SNA titers against BVDV and BHV1 were log2 transformed prior to analysis, but were back-transformed for reporting purposes. For each continuous outcome, a mixed generalized linear model for repeated measures was used to perform an ANOVA for comparison of differences among treatment groups. Fixed effects included in the model were treatment group (A, B, C, D, and E), sample acquisition day (ie, time), and the interaction between treatment group and time. Random effects included in the model were animal (to account for repeated measures) and a random residual. Multiple pairwise comparisons between treatment groups or various times were performed only if the overall fixed effect for treatment group or the interaction between treatment group and time was significant (P < 0.05). The Dunnett test was used to compare outcomes between the control group (group A) and the other treatment groups.

A multivariable repeated-measures ANOVA was used to compare the mean clinical evaluation scores for abnormal respiration, depression, and fecal consistency among the treatment groups following the challenge exposure. The proportions of calves with abnormal clinical scores (≥1) for respiration, depression, and fecal consistency were compared among treatment groups by use of logistic regression and a χ² test. Odds ratios were calculated by comparing the proportion of calves with abnormal clinical scores in each of the vaccinated groups (B, C, D, and E) with the proportion of calves with abnormal clinical scores within the control group (A; referent). For nominal variables (virus isolation, qPCR, and RTfPCR results and the proportion of calves that seroconverted [had ≥4-fold increase in SNA titers during the challenge exposure] against BVDV and BHV1), logistic regression was performed and a χ² test was used to compare the results among the treatment groups. All analyses were performed with commercially available statistical software; and values of P < 0.05 were considered significant.

Results

BVDV and BHV1 challenge exposure

Bovine viral diarrhea virus was detected in serum samples and nasal swab specimens obtained from all 6 PI cattle on days 0 (initiation of challenge exposure) and 28 (end of challenge exposure). The BVDV titers ranged from 1.1 × 10³ CCID₅₀/mL to 6.2 × 10³ CCID₅₀/mL in serum samples and from 6.2 × 10³ CCID₅₀/mL to 1.1 × 10⁴ CCID₅₀/mL in nasal swab specimens. None of the PI cattle developed clinical signs of BVDV or BHV1 infection while commingled with the study calves. Bovine viral diarrhea virus was isolated in serum, WBCs, and nasal swab specimens obtained from the calves in the 5 treatment groups, which suggested that BVDV was successfully transmitted among the commingled calves.

Bovine herpesvirus type 1 was detected by qPCR assay in nasal swab specimens obtained from 7 of the 8 calves experimentally inoculated with BHV1 on days 3, 6, 7, and 14, but was not detected in any of the serum samples obtained from the experimentally inoculated calves. However, BHV1 DNA was detected by qPCR assay in nasal swab specimens obtained from the calves in the 5 treatment groups, which suggested that, similar to BVDV, BHV1 was transmitted among the commingled calves.

Clinical scores, rectal temperature,
and body weight

Adverse vaccine reactions were not observed in any of the calves assigned to the 5 treatment groups (A, B, C, D, and E). None of the calves in the 5 treatment groups developed severe clinical signs (score, ≥3) of disease or died during or after the BVDV and BHV1 challenge exposure. The number of calves that were assigned an abnormal clinical score for respiration, depression, and fecal consistency did not differ significantly among the treatment groups (Table 1). The mean rectal temperature did not differ significantly among the treatment groups during the challenge exposure (Figure 1). The body weight of the calves in the treatment groups increased throughout the duration of the study (Figure 2); however, the mean body weight did not differ significantly among the treatment groups on day 45 (weaning and vaccination), 0, 28, or 56.

Virus detection

All of the calves assigned to the 5 treatment groups had negative virus isolation results for BVDV and BHV1 on day 0. Bovine viral diarrhea virus was isolated from WBC or serum samples (ie, viremia) of 9 calves in group A between days 3 and 14, 1 calf in group B on day 6, 2 calves in group C on day 14, and 4 calves in group D between days 6 and 10; BVDV was not isolated from any of the calves of group E. Bovine viral diarrhea virus was not isolated in any samples obtained on days 21 and 28. The number of calves with BVDV viremia in group A was significantly greater than that in groups B, C, and E on days 6, 7, 8, 9, 10, and 14.
Compared with the calves in group D, the number of calves with BVDV viremia in group A did not differ significantly on days 6, 7, 8, and 9 but was significantly greater on days 10 and 14.

Calves in which BVDV RNA was detected in nasal swab specimens by qPCR assay were considered BVDV shedders. During the challenge exposure, BVDV was shed by 5 calves in group A on days 6 through 21; 2 calves in group B on days 9 and 28, respectively; 1 calf in group C on day 8; 3 calves in group D on days 8, 9, and 21, respectively; and 0 calves in group E. The number of BVDV shedders in group A was significantly (P = 0.01) greater than that for group E, but did not differ significantly from that in groups B, C, and D. In group A, all 5 calves identified as BVDV shedders were also viremic at some point during the challenge exposure; however, BVDV viremia was not detected in the BVDV shedder in group C and at least one of the BVDV shedders in both groups B and D.

Bovine herpesvirus type 1 was not isolated in any serum samples. However, BHV1 DNA was detected in nasal swab specimens from 3 calves in group A on days 7, 9, and 14, respectively; 3 calves in group B on days 6 and 9; 1 calf in group C on days 9 and 14; 2 calves in group D on days 3 and 7, respectively; and 1 calf in group E on day 7. The number of calves that shed BHV1 did not differ significantly among the treatment groups.

Table 1—Number of calves within each of 5 treatment groups that were assigned an abnormal clinical score (≥ 1) for respiration, depression, and fecal consistency at least once in a 28-day period during which they were commingled with 6 cattle PI with BVDV and 8 cattle that were acutely infected with BHV1 (challenge exposure).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Respiration</th>
<th>Depression</th>
<th>Fecal consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with abnormal score</td>
<td>OR</td>
<td>No. with abnormal score</td>
</tr>
<tr>
<td>A</td>
<td>11</td>
<td>Referent</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>0.9</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>0.9</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>0.9</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>0.7</td>
<td>7</td>
</tr>
</tbody>
</table>

Calves were randomly assigned to 1 of 5 groups and administered PBSS (2 mL, SC; group A [control]; n = 11) or 1 of 4 commercially available MLV vaccines that contained antigens against BHV1, BVDV types 1 (BVDV1) and 2 (BVDV2), PI3V, and BRSV (2 mL, SC; groups B [11], C [10], D [11], and E [11]) 45 days prior to the introduction of PI and BHV1-infected cattle. Calves were visually evaluated on days 0 (initiation of challenge exposure), 3, 6, 7, 8, 9, 10, 14, 21, and 28 (end of challenge exposure) and assigned 3 scores on a scale of 0 to 3 to characterize abnormal respiration, depression, or fecal consistency. Calves were classified as having normal respiration (no nasal discharge; 0) or serous (1), mucoid (2), or mucopurulent (3) nasal discharge. Depression was classified as none (0), mild (1), moderate (2), or severe (3). Fecal consistency was classified as normal (0), pasty (1), runny (2), or severe diarrhea (3). The respective ORs represent the odds of calves with abnormal clinical scores from a vaccinated group (B, C, D, or E) relative to the odds of calves with abnormal clinical scores from the control group (A; referent). Within a condition (respiration, depression, fecal consistency), the number of calves that were assigned an abnormal clinical score (≥ 1) did not vary significantly among the treatment groups.

**Figure 1**—Mean ± SEM rectal temperatures for calves that were administered PBSS (2 mL, SC; group A [control]; n = 11; black line) or 1 of 4 commercially available MLV vaccines that contained antigens against BHV1, BVDV types 1 (BVDV1) and 2 (BVDV2), PI3V, and BRSV (2 mL, SC; groups B [11], C [10], D [11], and E [11]) 45 days prior to the introduction of PI and BHV1-infected cattle. Calves were weaned and administered the assigned treatment on day –45. The challenge exposure was initiated on day 0 and ended on day 28. Calves were monitored through day 56.

**Figure 2**—Mean body weight for the calves of groups A (black bars), B (red bars), C (green bars), D (purple bars), and E (blue bars) of Figure 1 at various times during the observation period. See Figure 1 for remainder of key.

**Genotype of BVDV isolates**

Alignment of the consensus sequences for BVDV isolates identified by RT-nPCR assay in WBC samples ob-
tained from calves assigned to the treatment groups revealed > 95% identity with the 5′ untranslated region of the consensus sequences for the BVDV isolates obtained from the 6 PI cattle. Bovine viral diarrhea virus type 1a was identified in 2 calves from group A and 3 calves from group D, BVDV1b was identified in 5 calves from group A, and BVDV2 was identified in 2 calves each of groups A, C, and D and 1 calf from group B.

SNA titers

On day −75 (median calf age, 40 days), the geometric mean SNA titers against BVDV1a, BVDV2, and BHV1 did not differ significantly among the 5 treatment groups (Table 2). Those titers were likely the result of passive transfer of maternally derived antibodies, which suggested that the extent of passive transfer of maternally derived antibodies was similar among all calves. For all treatment groups, the geometric mean SNA titers against BVDV1a, BVDV2, and BHV1 just prior to vaccination on day −45 were significantly decreased from those on day −75, which was indicative of the decay of maternally derived antibodies. On day 0 (45 days after vaccination and immediately prior to challenge exposure), the geometric mean SNA titers against BVDV1a and BVDV1b for calves in the groups (B, C, D, and E) that received an MLV vaccine were significantly greater than those for calves in the unvaccinated control group (group A); however, the geometric mean SNA titer against BVDV2 for calves in group E was significantly greater than that for the other 4 groups, and the geometric mean SNA titer against BHV1 did not differ significantly among the 5 treatment groups. On day 28 immediately after challenge exposure, the geometric mean SNA titer against BVDV1a for calves in groups B, C, and E was significantly greater than that for calves in groups A and D, and the geometric mean SNA titer against BVDV2 for group E was significantly greater than that for the other 4 groups, whereas the geometric mean SNA titers against BVDV1b and BHV1 did not differ significantly among the 5 treatment groups. For all treatment groups, the geometric mean SNA titer against BHV1 appeared to be unaffected by vaccination and decreased throughout the observation period (days −75 through 28) in a manner consistent with the decay of maternally derived antibody.

Table 2—Geometric mean (95% confidence interval) SNA titers against BVDV1a, BVDV1b, BVDV2, and BHV1 for the calves of Table 1 at various times before and immediately after the challenge exposure.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Group</th>
<th>Study day</th>
<th>−75 (95% CI)</th>
<th>−45 (95% CI)</th>
<th>0 (95% CI)</th>
<th>28 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV1a A</td>
<td>77 (14.9–398.9)</td>
<td>19.3 (7.3–95.7)</td>
<td>13.1 (3.3–51.6)</td>
<td>46.5 (19–113.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>93 (24.7–349)</td>
<td>32 (9–112.9)</td>
<td>388.8 (147–390.7)</td>
<td>657.1 (284–1,520)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>128 (28.6–568)</td>
<td>9.4 (2.9–30.7)</td>
<td>47.3 (17–138)</td>
<td>91.9 (34–230)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>67.6 (9.9–464.6)</td>
<td>9.2 (3.3–27.9)</td>
<td>101.8 (39–271)</td>
<td>315.6 (124–798)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>77 (15.5–382.6)</td>
<td>34.0 (7.7–91.4)</td>
<td>186.1 (109–315)</td>
<td>792.35 (436–458.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BVDV1b A</td>
<td>10.9 (3.7–31.7)</td>
<td>2.2 (1.2–8.6)</td>
<td>6.2 (2.2–16.9)</td>
<td>68.1 (17.2–268.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>21 (8.1–51)</td>
<td>128 (88.6–183.5)</td>
<td>423.5 (147–1,036)</td>
<td>328.5 (122.8–879.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14.9 (5.1–42.8)</td>
<td>18.4 (6.3–53)</td>
<td>337.8 (179–630)</td>
<td>186.0 (68.1–508.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>14.9 (5.1–42.8)</td>
<td>18.4 (6.3–53)</td>
<td>337.8 (179–630)</td>
<td>186.0 (68.1–508.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>14.9 (5.1–42.8)</td>
<td>18.4 (6.3–53)</td>
<td>337.8 (179–630)</td>
<td>186.0 (68.1–508.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BVDV2 A</td>
<td>145 (25.1–837.5)</td>
<td>35 (9–128)</td>
<td>2.7 (1.2–6)</td>
<td>82 (29.2–158)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>99 (25.6–382.6)</td>
<td>18 (6–53)</td>
<td>56 (24–132.5)</td>
<td>328.5 (152.3–699.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>111.4 (29.6–415.8)</td>
<td>32 (10.6–95.6)</td>
<td>37.9 (12–114)</td>
<td>256 (122.2–1,045)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>111.4 (29.6–415.8)</td>
<td>32 (10.6–95.6)</td>
<td>37.9 (12–114)</td>
<td>256 (122.2–1,045)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>111.4 (29.6–415.8)</td>
<td>32 (10.6–95.6)</td>
<td>37.9 (12–114)</td>
<td>256 (122.2–1,045)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHV1 A</td>
<td>132 (3.9–446)</td>
<td>6.6 (1.9–22.3)</td>
<td>2.7 (1–6.6)</td>
<td>2.7 (1–6.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17 (6.5–51.6)</td>
<td>7.4 (2.8–20)</td>
<td>4 (2–7.9)</td>
<td>2.2 (1–4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>18.4 (6.3–53)</td>
<td>10.5 (3.8–29.2)</td>
<td>2.3 (1.2–4.2)</td>
<td>2.2 (1.2–4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8.5 (3.7–30.2)</td>
<td>5.8 (1.75–19.4)</td>
<td>2.9 (1.2–6.9)</td>
<td>2.2 (1–4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>10.2 (3.2–32.9)</td>
<td>5 (1.8–11.4)</td>
<td>4.5 (1.7–8)</td>
<td>2.5 (1–6.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calves were weaned and administered the assigned treatment on day −45. The challenge exposure was initiated on day 0 and ended on day 28. *Within a virus and study day, values with different superscript letters differ significantly (P < 0.05); an absence of symbols indicates that values did not differ significantly among the treatment groups on that day.

See Table 1 for remainder of key.
For the purpose of this study, seroconversion was defined as a ≥4-fold increase in SNA titer against a particular viral pathogen following vaccination (day –45). None of the calves in group A seroconverted to any of the viruses evaluated, and none of the calves in groups B, C, D, and E seroconverted to BHV1. Eight calves in group B, 7 calves in group C, 3 calves in group D, and 5 calves E seroconverted to BVDV1a, and the number of calves that seroconverted to BVDV1a in groups B, C, and E was significantly greater than that in group D. Eight calves in group B, 6 calves in group C, 3 calves in group D, and 7 calves in group E seroconverted to BVDV1b, and, similar to BVDV1a, the number of calves that seroconverted to BVDV1b in groups B, C, and E was significantly greater than that in group D. Eight calves in group B, 3 calves in group C, 3 calves in group D, and 9 calves in group E seroconverted to BVDV2, and the number of calves that seroconverted to BVDV2 in groups B and E was significantly greater than that in groups C and D.

WBC and platelet counts
The mean WBC count decreased significantly (P < 0.001) from days 0 to 14 for all treatment groups (Figure 4). On day 0, the mean WBC count for calves in group E was significantly (P = 0.023) lower than that for calves in group A on day 0. †For all groups, the mean WBC count on day 14 was significantly (P < 0.001) lower than the corresponding mean WBC count on day 0. See Figure 1 for remainder of key.

Discussion
Stress associated with weaning, transport, and exposure to respiratory pathogens during commingling is an important risk factor for the development of BRDC in beef calves. In the present study, beef calves were weaned early (median age, 95 days vs the more traditional 180 to 240 days), vaccinated, transported to a research feedlot, and commingled with PI and BHV1-infected cattle simultaneously for 28 days in an attempt to simulate the environmental and management risk factors for BRDC frequently encountered by early-weaned beef calves. None of the calves in the present study developed clinical signs of severe respiratory disease or died during the observation period, and the clinical evaluation scores and mean rectal temperature, body weight, and WBC and platelet counts generally did not differ significantly among the treatment groups at the times evaluated. This finding suggested that maternally derived immunity to BVDV and BHV1 provided the calves protection against clinical disease when they were exposed to PI cattle and cattle acutely infected with BHV1.

The challenge exposure model used in this study resulted in successful transmission of BVDV as evident by the fact that several of the calves assigned to the treatment groups developed BVDV viremia and shed the virus. Also, the nucleotide sequences of the BVDV isolates obtained from viremic calves had >95% homology with the nucleotide sequences of the BVDV isolates obtained from the 6 PI cattle with which they were commingled. Unfortunately, BHV1 transmission during the challenge exposure was less than optimal as evidenced by the fact that the virus was not isolated from any serum samples and BHV1 DNA was detected in the nasal swab specimens from only 10 of the 54 calves assigned to the treatment groups. It is possible that the quantity of BHV1 shed by the 8 experimentally inoculated and acutely infected calves was not sufficient to sustain virus transmission to a large number of calves. Also, peak shedding of BHV1 typically occurs 5 to 6 days following experimental inoculation of naïve calves with the virus.9,10,19 In the present study, the 8 calves experimentally infected with BHV1 were inoculated 7 or 5 days prior to initiation of the challenge exposure (day 0); thus, peak shedding of BHV1 by those calves might have occurred prior to commingling with the calves assigned to the treatment groups. Additionally, all of the calves assigned to the treatment groups had maternally derived antibodies prior to the challenge exposure. It is possible that maternally derived antibodies against BHV1 effectively neutralized the virus9,13 thereby minimizing its transmission within the study population.

Despite the absence of severe clinical signs of disease caused by BVDV in the calves of the present study, the number of calves that were presumably infected, became viremic, and shed BVDV varied significantly among the treatment groups at any time.
cantly among the 5 treatment groups. Interestingly, not all of the calves that became viremic after being vaccinated with an MLV vaccine (ie, calves in groups B, C, and D) shed BVDV in their nasal secretions, and those that did shed BVDV did not do so until day 21 or 28 of the challenge exposure, which suggested that the BVDV detected in the nasal secretions of those calves might have been the result of contamination from continuous exposure to PI cattle rather than an actual infection. The calves in groups B, C, and E had fairly high SNA titers against BVDV1a and BVDV1b, and BVDV2 prior to initiation of the challenge exposure, and we believe those anti-BVDV antibodies played a critical role in reducing the incidence of BVDV viremia and shedding in those calves when commingled with PI cattle. Results of multiple studies indicate that, following experimental exposure to BVDV, calves with low anti-BVDV SNA titers (geometric mean titer, < 64) are at greater risk of developing clinical disease and viremia and shedding the virus than are calves with high anti-BVDV SNA titers.

In the present study, all dams of the calves assigned to the treatment groups were vaccinated prior to breeding with the same MLV vaccine that was administered to the calves of group D. Following vaccination, the geometric mean SNA titers against BVDV1a and BVDV1b for group D were lower than those for groups B, C, and E, and the number of calves that became viremic or shed BVDV was higher for group D than that for groups B, C, and E. Because the calves in group D were vaccinated with the same vaccine as their dams, we believe that the maternally derived anti-BVDV antibodies in those calves might have partially inactivated the vaccine virus and limited the induction of antibodies against BVDV. Thus, as the maternally derived anti-BVDV antibodies decayed, the calves in group D were at greater risk of becoming infected with BVDV than were the calves in the other groups that were vaccinated with a different MLV vaccine and had higher serum concentrations of anti-BVDV antibodies. However, this is strictly supposition and further investigation is warranted.

Age and the serum concentration of maternally derived antibodies affect the humoral response of young calves following administration of MLV vaccines. Minimal titers of maternally derived antibodies against BHV1 can interfere with anti-BHV1 antibody production and seroconversion in vaccinated calves. This might explain why the SNA titers against BHV1 in the calves of the present study continued to decrease after vaccination.

Results of other studies indicate that protection against clinical disease, viremia, and viral shedding for calves with maternally derived antibodies that were vaccinated against BVDV with an MLV vaccine and subsequently exposed to a virulent strain of BVDV is associated with vaccinal priming of specific T lymphocyte populations that induce an anamnestic cell-mediated response with or without the presence of maternally derived anti-BVDV antibodies. However, in another study, 12 of 15 calves that were vaccinated against BVDV with an MLV vaccine at 3 days old and then exposed to a virulent strain of BVDV at 7 to 9 months old developed viremia, although none developed severe clinical disease. This suggests that administration of an MLV vaccine against BVDV to young calves to prime the T lymphocyte response does not always prevent those calves from becoming viremic and potentially shedding the virus when they are subsequently exposed to BVDV. Interestingly, in the present study, groups B, C, and E, which had the highest geometric mean titers against all 3 types of BVDV, had the lowest number of calves that became viremic or shed BVDV during the challenge exposure. Thus, it appeared that the humoral response induced by an MLV vaccine was as important in providing calves protection against BVDV as was the anamnestic cell-mediated response resulting from vaccinal priming of T lymphocytes.

Results of the present study indicated that administration of any of 4 commercially available multivalent MLV vaccines that contained antigens against BHV1, BVDV1, BVDV2, Pi3V, and BRSV to early-weaned beef calves with maternally derived antibodies prevented clinical disease, resulted in an increase in SNA titers against BVDV, and reduced the incidence of BVDV viremia and shedding. The reduction of viremia and BVDV shedding in young calves following vaccination with an MLV vaccine is associated with a reduction in BVDV transmission and exposure within a population. Thus, administration of multivalent respiratory MLV vaccines to early-weaned beef calves may help minimize the effects of BVDV in those calves when they arrive at stocker or backgrounding operations or feedlots, which should decrease the overall incidence of BRDC and its associated economic costs and improve animal health on those operations.

Acknowledgments

All procedures described within this study were performed at Auburn University College of Veterinary Medicine, Auburn, AL 36849.

Supported by the Alabama Agricultural Experimental Station, Auburn, Ala.

The authors thank Dr. Soren Rodning, Mr. George Fincher, and Mr. Steven Ledbetter for technical assistance.

This manuscript represents a portion of the dissertation submitted by Dr. Chamorro to the Auburn University Graduate School as partial fulfillment of the requirements for a Doctor of Philosophy degree.

Footnotes

a. BoviShield Gold 5, Zoetis Inc, Florham Park, NJ.
b. SAS, SAS Institute Inc, Cary, NC.
c. Microsoft Excel 2010, Microsoft Corp, Redmond, Wash.
d. BRD-Shield, Novartis Animal Health US Inc, Larchwood, Iowa.
e. Express 5, Boehringer Ingelheim, Ridgefield, Conn.
f. Viralign 6, Elanco Animal Health, Greenfield, Ind.
g. Inforce 3, Zoetis Inc, Florham Park, NJ.
h. VR-864, ATCC, Manassas, Va.
i. True-test Inc, Mineral Wells, Tex.
j. High Pure PCR Template preparation kit, Roche Pharmaceuticals, Branchburg, NJ.
k. iTag Universal SYBR Green One-step kit, Bio-Rad Laboratories Inc, Hercules, Calif.
References