The titers, duration, and residual clinical protection of passively transferred nasal and serum antibodies are similar among beef calves that nursed colostrum from vaccinated or unvaccinated dams and were challenged experimentally with bovine respiratory syncytial virus at three months of age

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doi.org/10.2460/ajvr.22.07.0118

OBJECTIVES
To compare initial titers, duration, and residual clinical protection of passively transferred bovine respiratory syncytial virus (BRSV) nasal immunoglobulin (Ig) G-1 and IgA, and serum neutralizing (SN) antibodies.

ANIMALS
40 three-month-old beef steers born either to unvaccinated or vaccinated cows.

PROCEDURES
During the last trimester of gestation, cows were assigned randomly to either vaccinated or unvaccinated groups. Calves were grouped on the basis of whether they nursed colostrum from unvaccinated dams (NO-VACC group; n = 20) versus dams vaccinated with 2 doses of an inactivated BRSV vaccine (VACC group; n = 20). At 3 months of age, calves were challenged with BRSV. Respiratory signs were scored. Nasal BRSV IgG-1 and IgA and SN antibodies were compared before and after the challenge. The presence of BRSV in nasal secretions was evaluated by reverse transcription-PCR assays.

RESULTS
Respiratory scores after BRSV challenge were similar between treatment groups. Nasal BRSV IgG-1 and SN antibodies were significantly greater in VACC calves at 48 hours of life; however, by 3 months of age, titers had decayed in both groups. Nasal BRSV IgA titers were minimal after colostrum intake and before the BRSV challenge, and increased in both groups after the challenge. The NO-VACC group had a significantly greater probability of shedding BRSV compared with VACC calves.

CLINICAL RELEVANCE
At 3 months of age, titers of passively transferred BRSV antibodies in VACC and NO-VACC calves had decayed to nonprotective levels. Calves born to vaccinated dams had a decreased probability of BRSV shedding; however, this was not related to differences in SN or nasal BRSV antibody titers.

Bovine respiratory syncytial virus (BRSV) plays an important etiologic role for respiratory disease in calves younger than 6 months of age and can be an important contributor to preweaning beef calf pneumonia in US cow-calf operations. Preweaning or “summer” beef calf pneumonia usually occurs in calves between 2 and 4 months of age. The vast majority of studies describing BRSV-associated calf morbidity and mortality rates in North America involve dairy calves, with a minimal number of studies of beef calves. However, high morbidity and mortality rates due to respiratory disease resulting from BRSV infection in beef calves have been reported in European countries. Vaccination of calves
between 1 and 30 days of age has become a standard strategy among producers and veterinarians to prevent calf morbidity and mortality rates associated with BRSV infection; however, successful reduction of clinical signs of disease after calf vaccination has been inconsistent in studies of naturally occurring and experimentally induced BRSV infection.\textsuperscript{2,5,9,11,12} The presence of colostrum-derived serum antibodies in young calves may suppress respiratory tract and circulatory antibody responses after experimental infection or vaccination and could explain the inconsistencies observed with vaccination efficacy.\textsuperscript{13,14} Despite interfering with vaccine-induced local (respiratory tract) and systemic humoral responses, moderate to low levels of serum neutralizing (SN) antibodies derived from colostrum protect young calves against clinical disease after acute infection with respiratory viruses such as bovine viral diarrhea virus (BVDV) 1 and BVDV 2, bovine herpesvirus 1 (BHV-1) and BRSV.\textsuperscript{15–17} Cell-mediated immune responses in previously vaccinated calves may also contribute to protection,\textsuperscript{18,19} but these are reported less frequently.

Immunoglobulin G-1 (IgG-1) is the most prevalent immunoglobulin in colostrum and is transferred preferentially to the respiratory tract of calves after colostrum intake.\textsuperscript{20} Results from one study suggested that colostrum-derived IgG-1 present in the upper respiratory tract of young lambs can prevent parainfluenza 3 virus replication.\textsuperscript{21} In contrast to IgG-1, the concentration of immunoglobulin A (IgA) in colostrum is minimal, and its transfer to the respiratory tract of calves as well as its role in clinical protection against acute viral infection has not been described.\textsuperscript{22} It is possible that high levels of BRSV IgG-1 transferred from colostrum to the respiratory tract reduces BRSV infection and clinical disease in young calves; however, the duration of colostrum-derived nasal IgG-1 and IgA—and their role in the clinical protection of calves against BRSV infection—have not been described. The first objective of this study was to compare the initial titers and duration of nasal BRSV IgG-1 and IgA and SN antibodies in calves born to vaccinated or unvaccinated dams. The second objective of this study was to determine whether residual clinical protection was afforded by passively transferred BRSV antibodies. We hypothesized that vaccination of pregnant dams would result in a greater transfer of colostrum-derived BRSV antibodies to their calves. In addition, we hypothesized that a greater initial titer of colostrum-derived BRSV antibodies would improve the residual clinical protection afforded by maternal immunity after experimental challenge with BRSV at 3 months of age.

### Materials and Methods

#### Experimental design

The Auburn University Institutional Animal Care and Use Committee (PRN No. 2019-3530) reviewed and approved all animal protocols in this study. Cows from a single 175-head cow-calf herd were enrolled in this randomized, controlled clinical trial during pregnancy diagnosis in summer 2019. At approximately 6.5 to 7.5 months of gestation, cows were stratified by age and assigned randomly to 2 different treatment groups. The unvaccinated group received 5 mL of 0.9% phosphate buffered saline SC (Veltix; Dechra Veterinary Products) 21 days apart. The vaccinated group received 2 doses of a multivalent inactivated-virus vaccine (Traingle10; Boehringer Ingelheim Animal Health USA Inc) containing BRSV 21 days apart according to the manufacturer’s recommendations. Approximately 1 month before calving, all cows were moved into a single pasture and monitored for parturition by on-farm personnel blinded to treatment allocation.

After calving, all calves nursed colostrum naturally from their respective dams and without assistance. By 24 hours of age, all male calves were weaned, identified (individual radio frequency electronic tag and regular ear tag), and castrated. After all calves had been born, a random sample of 20 calves each—born to vaccinated dams (VACC group; n = 20) or unvaccinated dams (NO-VACC group; n = 20)—were selected for the experimental portion of the study. The study calves remained with their dams in the same pasture until early weaning. One month prior to weaning, creep feeders were introduced to the pasture to allow calves to familiarize with concentrated feed. At 3 months of age, all calves were weaned abruptly and transported 200 miles to the North Auburn BVDV research unit for the BRSV challenge portion of the study. On arrival to the unit, calves were placed in a single pasture with ad libitum access to fresh hay, water, and concentrated feed. Five days after arrival, all calves were challenged with BRSV by intranasal nebulization as described later.

Blood samples from dams were collected before initial vaccination and at calving for virus neutralization antibody testing. At calving, maternal colostrum samples from each cow were assessed indirectly for immunoglobulin G (IgG) concentration using Brix refractometry (MA871 Digital BRIX refractometer; Milwaukee Instruments). Transfer of passive immunity was also evaluated indirectly in all calves by serum Brix refractometry at 48 hours of age. Blood and nasal secretion samples from calves were collected before and after BRSV challenge for antibody and virologic assays.

#### BRSV challenge

On day 0, each calf was challenged with 7 mL of a lung wash inoculum that had been expanded once in Marvin Darby bovine kidney (MDBK) cells and contained 1 X 10^4 tissue culture infectious dose (TCID50) of BRSV strain GA-1/mL by individual intranasal nebulization using an electronic nebulizer (Pulmo-Aide compressor nebulizer system; DeVilbiss Health Care) connected to a face mask (Era Equine Mask; BIOMEDTECH).

#### Clinical evaluation and sample collection

After arrival at the BVDV unit and before the experimental challenge with BRSV, on-farm personnel blinded to treatment allocation observed
calves daily and recorded clinical signs of disease (eg, cough, nasal discharge, diarrhea, etc.) and treatments in individual-calf notebook sheets. After BRSV challenge, clinical evaluation and scoring of calves was performed by a single veterinarian blinded to treatment group allocation on days 0, 4, 6, 8, 10, 14, 21, and 28 relative to BRSV challenge. Blood samples were collected at 48 hours and 30 days of life, and on days 0 and 28 after challenge for BRSV serum neutralization assays. Nasal secretion samples were collected at 48 hours and 30 days of life and on days 0, 4, 21, and 28 for BRSV IgG-1 and IgA determination. Additional nasal secretion samples were collected on days 0, 4, 6, 8, 10, 14, 21, and 28 to determine the presence of BRSV by the use of reverse transcription (RT)-PCR assays. Samples from each calf were labeled such that treatment allocation remained masked from personnel processing samples and performing the assays.

On sampling days, clinical signs such as depression, rectal temperature, respiratory rate, cough, and nasal discharge were evaluated, and a total respiratory score was assigned to each calf as described previously. Clinical scores were assigned in a scale of 0 to 3, where 0 was considered normal or absent of abnormalities and 3 was the most abnormal finding. Depression was scored from 0 (bright, alert, responsive) to 3 (obtunded, recumbent, nonresponsive), rectal temperature was scored from 0 (37.8 to 38.3°C) to 3 (> 39.4°C), respiratory rate was scored from 0 (respiratory rate < 30 respirations/min [rpm]) to 3 (respiratory rate > 100 rpm), cough was scored from 0 (none) to 3 (repeated spontaneous cough), and nasal discharge was scored from 0 (none or serous discharge) to 3 (purulent bilateral discharge). The sum of individual scores including rectal temperature, depression, respiratory rate, nasal discharge, and cough determined the presence of mild, moderate, or severe respiratory disease. Briefly, mild respiratory disease was determined by a sum of individual scores between 0 and 5, moderate respiratory disease was determined by a sum of scores between 6 and 10, and severe respiratory disease was indicated when the sum of scores was > 10. In addition to clinical evaluation, individual body weights were obtained at birth, on day 0 (BRSV challenge), and on days 14 and 28 after BRSV challenge using a portable livestock electric scale (Livestock Platform Scale; Brecknell) that was calibrated prior to and after each weighing.

**BRSV neutralizing antibodies in serum**

A virus neutralization assay for the detection of serum anti-BRSV antibodies was performed as described previously. Serum samples were thawed and heat-inactivated in a water bath at 55°C for 30 minutes; then, serial 2-fold dilutions (1:10 to 1:1,000) were made in 96-microwell flat-bottom plates, and 500 µL of 100 TCID50 BRSV suspended in minimum essential medium was added to all wells. For each dilution, 3 microwells were inoculated with equal volumes of virus culture media. After incubation at 37°C in 5% carbon dioxide for 1 hour, MDBK cell cultures were inoculated with minimum essential medium that included 7% bovine serum and an antimicrobial and antimycotic solution containing streptomycin, penicillin, and amphotericin B. The plates were then incubated for up to 2 weeks and monitored daily for the presence of cytopathic effects by microscopic evaluation. Antibody titers were then reported as the inverse of the lowest dilution of serum required to inhibit all cytopathic effects and were log2-transformed for statistical analysis.

**Determination of anti-BRSV IgG-1 and IgA in nasal secretions**

Bovine respiratory syncytial viral particles were inactivated with 2 µM binary ethylenimine, neutralized with sodium thiosulfate, and diluted 1:800 in carbonate bicarbonate buffer (pH, 9.5). The resulting solution was used to coat microwells of 96-well polystyrene plates. After coating, the plates were incubated overnight at 4°C and washed 3 times with PBS containing 0.05% polysorbate 20 (Tween 20; Sigma-Aldrich). After washing, 200 µL of PBSS containing 5% sheep serum albumin (Sigma-Aldrich) was added to each well for blocking, the plates were incubated at 37°C for 1 hour, and then were washed 3 times.

Vials containing nasal secretion samples were thawed and vortexed. Each sample was initially diluted 1:1 in Pluronic F127 (Sigma-Aldrich, St) and then diluted 1:100 in polysorbate 20 (Sigma-Aldrich). From this dilution, serial 2-fold dilutions were prepared up to 1:200 for IgG-1 and up to 1:1,600 for IgA, and each dilution was analyzed in triplicate (ie, each sample dilution was added to 3 wells). If the coefficient of variation among the 3 values was > 20%, the outlier value was removed, and the mean value of the 2 remaining samples was used in the calculation of the antibody titer. Samples with an optical density value that was too high to be measured accurately were tested again at a greater dilution. Samples with an optical density value that was too low to be measured accurately were tested again at a lower dilution, with the lowest dilution tested being 1:25 for IgA and 1:100 for IgG-1. In addition to the samples, each plate had 4 microwells containing the following: positive control, which was a nasal secretion sample from a known BRSV antibody positive calf diluted 1:100 in polysorbate 20; negative control, which was low-IgG fetal bovine serum (Sigma-Aldrich) diluted 1:100 in polysorbate 20; and blank, which was polysorbate 20 alone. For BRSV-specific IgA, horseradish peroxidase (HRP)-conjugated rabbit anti-bovine IgA (Bio-Rad) diluted 1:500 in an ELISA wash buffer (PBS + 0.05% Tween 20) and 2,2′-Azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-dihammonium salt (ABTS) substrate solution (ABTS; Sigma-Aldrich) were added to each well. For BRSV IgG-1, HRP-conjugated sheep anti-bovine IgG-1 (Bio-Rad) diluted 1:7,500 in an ELISA wash buffer (PBS + 0.05% Tween 20) and o-phenylenediamine dihydrochloride (OPD) substrate were added to each well. All plates were read by a plate reader set at a wavelength of 405 nm. Wells positive for anti-BRSV IgA and IgG-1 yielded a green (IgA) and yellow (IgG-1) product when the
bound peroxidase-conjugated rabbit anti-bovine IgA and sheep anti-bovine IgG-1 reacted with the ABTS and OPD substrates, respectively. Immunoglobulin A and IgG-1 titers were reported as the inverse of the last dilution that was ≥ 2 times the mean optical density value of the negative control.

**Determination of BRSV RNA in nasal secretions**

Real-time RT-PCR was performed in nasal secretion samples as previously described. Briefly, nasal secretion sample aliquots were subjected to RNA extraction using a commercially available reagent (RNAzol; Sigma-Aldrich) according to the manufacturer’s recommendations. Once extracted, the RNA templates were reverse-transcribed and amplified (qScript XLT One-Step RT-qPCR ToughMix, qScript; Sigma-Aldrich) using BRSV-specific primers and probes. All reactions were performed with a PCR platform (Light Cycler 480 II; Roche) and results were analyzed with the manufacturer’s software (Light Cycler 480 SW version 1.5; Roche).

**Statistical analysis**

Data were analyzed using statistical software (RStudio version 1.4.1717; Posit). The normality of the data was assessed using the Shapiro-Wilk test and examination of the residuals. Data were analyzed using generalized mixed-effects models with animal identifier as the random effect; immunoglobulin titers, virus neutralization titers, rectal temperatures, and body weights as dependent variables; and vaccination status and experiment time as independent variables. Post hoc familywise comparisons were performed using Tukey-Kramer with Bonferroni correction. Kaplan-Meier curves were generated to display BRSV shedding via nasal secretions over time for NO-VACC versus VACC calves. For nonparametric variables and proportions (ie, Brix values, clinical scores), group comparisons were performed using the Fisher exact test, χ² (categorical predictor variables, 2 groups), Kruskal-Wallis (categorical predictor variables, > 2 groups), and Wilcoxon rank-sum test (to compare medians between 2 independent populations). For all analyses, significance was set at P < .05.

**Results**

**Transfer of passive immunity and clinical outcomes**

The median of maternal colostrum Brix at calving was greater in NO-VACC cows (28.1%) compared with VACC cows (26.4%); however, this difference was not statistically significant (P = .28). Similarly, the median serum Brix reading at 48 hours of life was greater in calves that nursed colostrum from NO-VACC dams (10.9%) compared with calves that nursed colostrum from VACC dams (10.4%); however, this difference was not statistically significant (P = .24).

Table 1—Descriptive statistics of body weight and clinical scores after experimental challenge with bovine respiratory syncytial virus (BRSV) of calves from a single herd that nursed colostrum from dams vaccinated with 2 doses of an inactivated BRSV vaccine (VACC group) versus calves that nursed colostrum from unvaccinated dams (NO-VACC group).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NO-VACC (n = 20)</th>
<th>VACC (n = 20)</th>
<th>P value</th>
<th>SMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg (median [IQR])</td>
<td>112.72 [71.58–124.74]</td>
<td>110.00 [52.23–123.72]</td>
<td>.77</td>
<td>0.04</td>
</tr>
<tr>
<td>Rectal temperature, °C (median [IQR])</td>
<td>38.9 [38.5–39.3]</td>
<td>38.4 [38.4–39.3]</td>
<td>.90</td>
<td>0.08</td>
</tr>
<tr>
<td>Rectal temperature score, 0–3 points (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30 (18.8)</td>
<td>31 (20.3)</td>
<td>.79</td>
<td>0.12</td>
</tr>
<tr>
<td>1</td>
<td>39 (24.4)</td>
<td>37 (24.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>63 (39.4)</td>
<td>53 (34.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28 (17.5)</td>
<td>32 (20.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression score, 0–2 points (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>138 (86.2)</td>
<td>138 (90.2)</td>
<td>.40</td>
<td>0.15</td>
</tr>
<tr>
<td>1</td>
<td>21 (13.1)</td>
<td>15 (9.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 (0.6)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough score, 0–3 points (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>160 (100.0)</td>
<td>158 (98.8)</td>
<td>.48</td>
<td>0.16</td>
</tr>
<tr>
<td>1</td>
<td>2 (1.2)</td>
<td>3 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal secretion score, 0–3 points (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>127 (79.4)</td>
<td>126 (82.4)</td>
<td>.20</td>
<td>0.25</td>
</tr>
<tr>
<td>1</td>
<td>20 (12.5)</td>
<td>21 (13.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11 (6.9)</td>
<td>3 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 (1.2)</td>
<td>3 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical scores (median [IQR])</td>
<td>2.00 [1.00–3.00]</td>
<td>2.00 [1.00–3.00]</td>
<td>.36</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Data were analyzed using generalized mixed-effects models. Data are reported as number and percentage of calves unless otherwise noted. IQR = Interquartile range. SMD = Standardized median difference.

*0 point, 37.8 to 38.3 °C; 3 points, > 39.4 °C. *0 point, bright, responsive; 3 points, recumbent, nonresponsive. *0 point, none; 3 points, repeated spontaneous cough. *0 point, none or serous; 3 points, purulent bilateral.
Clinical signs of disease were not observed in calves during the preweaning period and before the BRSV challenge. After the challenge, group VACC calf was found dead in the pasture on day 4. Necropsy and histopathologic evaluation of this calf revealed free gas bloat as the cause of death, but no respiratory tract lesions. Signs of respiratory disease such as tachypnea, cough, nasal discharge, and depression were not different between VACC and NO-VACC calves after the challenge (Table 1). The total respiratory score after the BRSV challenge was mild, and no evidence of statistical significance was detected between treatment groups (Figure 1). The proportion of calves that developed fever (rectal temperature > 39.7 °C) after the experimental challenge with BRSV was numerically greater in the NO-VACC group (20/20 [100%]) compared with the VACC group (15/20 [78%]); however, this difference was not statistically significant (P = .1).

There was a significant effect of time on the mean individual body weights within treatment groups as all calves gained weight from birth to weaning (P < .01 for all time-point comparisons within each group; Supplementary Table S5); however, no evidence of statistical significance was detected in the mean ± SEM individual body weights between groups throughout the study period (P > .99, for all comparisons between groups). For VACC and NO-VACC calves, the mean ± SEM average daily gain (ADG) from birth to weaning (0.75 ± 0.03 kg/d vs 0.71 ± 0.03 kg/d, respectively) was significantly greater (P = .01) compared with the mean ± SEM ADG from day 0 (challenge day) to the end of the study (0.35 ± 0.03 kg/d vs 0.31 ± 0.07 kg/d, respectively; Supplementary Table S6); however, this difference was not statistically significant (P > .5) between treatment groups.

**BRSV neutralizing antibodies in serum**

The mean ± SEM log₂ BRSV SN antibody titer before vaccination was not significantly different (P = .07) between VACC and NO-VACC cows (4.5 ± 0.39 vs 3.5 ± 0.55, respectively). At calving, the mean ± SEM log₂ BRSV SN antibody titer was significantly greater (P = .04) in VACC cows compared with NO-VACC cows (5.15 ± 0.59 vs 3.05 ± 0.49, respectively). At 48 hours of life, the mean log₂ BRSV serum antibody titer was significantly greater in VACC calves compared with NO-VACC calves. After the 48-hour time point, SN antibodies decreased within groups until the end of the study (Figure 2). The absolute mean of log₂ SN antibody titers was greater in VACC calves at 30 days of life, day 0 (challenge day), and day 28 compared with NO-VACC calves; however, these differences were not statistically significant at any time point between treatment groups (Supplementary Table S4).

**BRSV IgG-1 and IgA titers in nasal secretions**

At 48 hours of life, the mean nasal BRSV IgG-1 titer was significantly greater in VACC calves compared with NO-VACC calves (Table 2). However, after day 30 of life, BRSV IgG-1 titers were virtually absent in nasal secretion samples from calves in both groups for the remainder of the study period (Figure 2). In contrast, negative or low mean nasal BRSV IgA titers were detected in VACC and NO-VACC calves at 48 hours and 30 days of life, and the day of challenge. Nasal BRSV IgA titers increased progressively on days 4, 21, and 28 after BRSV challenge in calves from both groups, peaking at day 21; however, statistically significant differences between groups were not observed at any time point (Supplementary Tables S1 and S2).

**BRSV RT-PCR in nasal secretions**

After the BRSV challenge, a significantly greater (P = .01) proportion of NO-VACC calves (4/20 [20%]) were confirmed to be positive for BRSV on RT-PCR assay performed on nasal secretion samples compared with the proportion of VACC calves (1/20 [5%]). The median number of days on which nasal secretions were positive for BRSV on RT-PCR was 2 days for NO-VACC calves and 1 day for VACC calves.
calves. The risk of BRSV shedding based on RT-PCR results in nasal secretion samples after the challenge was significantly less ($P < .01$) in VACC calves compared with NO-VACC calves (Figure 3).

Figure 2—Mean ± SEM log$_{2}$-transformed serum neutralizing antibody titer (A), nasal secretion immunoglobulin (Ig) G-1 bovine respiratory syncytial virus (BRSV) antibody titer (B), and nasal secretion IgA BRSV antibody titer (C) for calves in the vaccinated (VACC; dashed line, triangles) group versus the unvaccinated (NO-VACC; solid line, circles) group as described in Figure 1 at baseline 48 hours after birth (BL-48h), baseline 1 month after birth (BL-1mo), and days 0 (challenge day), 21, and 28. Data were analyzed using generalized mixed-effects models. # = Results differed significantly ($P < .05$) between groups; distinct letters represent a significant ($P < .05$) difference between time points within participants of each group (vaccinated in bold type). Familywise multiple comparisons were performed using the Tukey-Kramer with Bonferroni correction. D = Day.

Table 2—Mean ± SEM serum neutralizing (SN), and nasal immunoglobulin (Ig) G-1 and IgA, bovine respiratory syncytial virus (BRSV) antibody titers at baseline (48 hours and 30 days of life) and after experimental challenge with BRSV (days 0 through 28) of calves in the vaccinated (VACC) versus unvaccinated (NO-VACC) groups described in Table 1.

<table>
<thead>
<tr>
<th>Test and time point</th>
<th>NO-VACC</th>
<th>VACC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRSV SN (mean log$_{2}$ ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hours of life</td>
<td>4.7 ± 0.5</td>
<td>6.2 ± 0.5</td>
<td>.04</td>
</tr>
<tr>
<td>30 days of life</td>
<td>4.2 ± 0.5</td>
<td>5.3 ± 0.6</td>
<td>.23</td>
</tr>
<tr>
<td>Day 0 (challenge day)</td>
<td>1.3 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 28</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>1.00</td>
</tr>
<tr>
<td>BRSV nasal IgG-1 (mean ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hours of life</td>
<td>14 ± 7.1</td>
<td>50 ± 13</td>
<td>.01</td>
</tr>
<tr>
<td>30 days of life</td>
<td>2.5 ± 1.7</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 0 (challenge day)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 4</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 21</td>
<td>1.2 ± 1.2</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 28</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>BRSV nasal IgA (mean ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hours of life</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>30 days of life</td>
<td>35 ± 15</td>
<td>90 ± 43</td>
<td>.10</td>
</tr>
<tr>
<td>Day 0 (challenge day)</td>
<td>50 ± 22</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 4</td>
<td>121 ± 41</td>
<td>142 ± 34</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 21</td>
<td>465 ± 96</td>
<td>410 ± 77</td>
<td>.97</td>
</tr>
<tr>
<td>Day 28</td>
<td>217 ± 59</td>
<td>211 ± 48</td>
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Figure 3—Kaplan-Meier curve of the cumulative probability of shedding bovine respiratory syncytial virus (BRSV; detected with reverse transcription-PCR assay) for calves in the vaccinated (dashed line) versus unvaccinated (solid line, circles) groups described in Figure 1 at time intervals 0 to 8, where each time interval represents days 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 14, 14 to 21, and 21 to 28, respectively, after BRSV challenge. Tick marks represent the end of each time period, each step represents detection events of BRSV shedding, and shading represents the respective 95% CI for the probability of shedding BRSV by calves in the vaccinated (red) versus unvaccinated (gray) groups.
Discussion

Maternally derived immunity against bovine respiratory viruses protects young calves against acute viral infection and disease. In the case of BRSV, results from a previous experimental BRSV infection study demonstrated reduction of clinical disease in neonatal calves with maternally derived immunity.\textsuperscript{15} Adequate colostrum IgG concentration and transfer is critical to provide effective and prolonged protection. In this study, the median Brix of colostrum in dams at calving (NO-VACC, 26.4%; VACC, 28.1%) was adequate and consistent with ideal IgG colostrum concentrations for beef cows.\textsuperscript{26} Similarly, the median serum Brix of calves at 48 hours of life (NO-VACC, 10.9%; VACC, 10.6%) was adequate and consistent with what is considered excellent transfer of passive immunity in beef calves.\textsuperscript{27} Results from previous studies\textsuperscript{28,29} have demonstrated that the greater the initial virus-specific serum antibody titer transferred from colostrum, the longer the duration of specific immunity in calves. Other studies\textsuperscript{30,31} suggest that vaccination of cows during the last trimester of gestation with inactivated-virus vaccines results in greater deposition of specific antibodies in maternal colostrum and greater initial BVDV and BHV-1 serum antibody titers in their calves. We demonstrated similar results in our study. Calves from groups VACC and NO-VACC had titers of serum and nasal BRSV antibodies transferred from colostrum at 48 hours, but VACC calves demonstrated significantly greater levels of SN and nasal BRSV IgG-1 antibody titers compared with NO-VACC calves. The differences in the initial local and systemic-specific BRSV antibody titers between calves in the 2 groups was likely the result of vaccination of cows during gestation. A greater proportion of the total passively transferred IgG-1 in VACC calves corresponded to the BRSV IgG-1 product of vaccination of their dams. Consequently, it is possible that IgG-1 specific to other infectious agents (ie, bacterial pathogens) was less in VACC calves compared with NO-VACC calves because the total IgG transfer was apparently similar between groups based on serum Brix results. Results from previous studies\textsuperscript{28,29} suggest that total serum IgG concentration at 24 to 48 hours is not an accurate predictor of the transfer of pathogen-specific passive immunity in calves. In our study, calves from the VACC and NO-VACC groups had low levels of BRSV SN antibody titers on the day of challenge, and despite a small numeric difference, clinical signs of respiratory disease after the challenge were not different between groups. It is unlikely that the low titers of BRSV SN antibody titers observed on the day of the challenge played a significant role on clinical protection or reduced nasal shedding in VACC calves. Previous studies\textsuperscript{31,32} suggest that calves with log\textsubscript{2} BRSV SN antibody titers of 2 to 4 at the time of challenge usually develop significant respiratory signs and nasal shedding after BRSV challenge. Although viremia and nasal shedding are reduced in calves with moderate SN antibodies against BVDV,\textsuperscript{16,17} SN antibody titers against BRSV do not reliably predict BRSV shedding or the extent of clinical disease.\textsuperscript{5,9} In addition, nasal BRSV shedding in study calves was determined by RT-PCR, which does not assess viability nor infectivity of BRSV. Virus isolation assays to confirm shedding would have been ideal to determine whether BRSV found in the nasal secretions of calves was viable and infective. Factors such as upper and lower respiratory tract innate immune responses, specific BRSV antibody titers in the lung, and cell-mediated immunity, which were not evaluated in our study, could have contributed to the reduction of BRSV shedding observed in VACC calves; however, it is possible that the absence of significant clinical disease and nasal shedding observed in calves in our study corresponded to a reduced efficacy of our BRSV challenge model. In vitro passage of BRSV through cell cultures before the challenge reduces the virulence of BRSV, and in this case it could have impaired viral fitness, replication, and dissemination to the lungs.\textsuperscript{33,34}

The rapid decay of nasal BRSV IgG-1 and the low levels of nasal BRSV IgG-1 before the BRSV challenge suggest that after 1 month of age, nasal BRSV IgG-1 and IgA derived from colostrum likely do not play a significant role in the clinical protection of calves against acute BRSV infection. We speculate that nasal BRSV IgG-1 transferred from colostrum may play an important role in clinical protection against viral infection and response to vaccination in calves younger than 1 month of age. Unfortunately, in our study, the levels of nasal BRSV IgG-1 were not evaluated at additional time points during the first month of age. The dynamics of IgG-1 transferred from colostrum into the respiratory tract (upper and lower) and its role on clinical protection and response to vaccination should be a matter of future investigation. Nasal BRSV IgA increased similarly in all calves after the experimental BRSV challenge, reaching peak levels at day 21 postinfection. This is consistent with postexposure or postvaccination studies\textsuperscript{13,14} in BRSV-naive calves in which nasal BRSV IgA was not detected before 8 to 10 days postinfection or postvaccination. The role of nasal BRSV IgA in the protection of calves against BRSV infection and shedding is inconsistent in the literature. Although in some studies\textsuperscript{14} the presence of BRSV IgA in the upper respiratory tract was related to a reduction of clinical disease and nasal shedding, results from other studies\textsuperscript{5,9} show a reduction of clinical disease and shedding in the absence of a significant increase in BRSV IgA responses.

Colostrum-derived local and systemic BRSV antibodies were initially greater in VACC calves; however, similar to NO-VACC calves, their titers decayed to nonprotective levels by 3 months of age and lacked apparent residual clinical protection from the BRSV challenge. Based on our results, we conclude that it is unlikely that local and systemic immunity transferred passively from colostrum can provide effective clinical protection against BRSV infection in beef calves 3 months of age or older. Therefore, vaccination of beef calves at a younger age still may be necessary to reduce morbidity and mortality rates caused by BRSV.
infection in cow-calf herds in which this pathogen contributes to the typical presentation of preweaning pneumonia between 2 and 4 months of age. We speculate that scheduling BRSV vaccination protocols for calves with adequate levels of passive immunity should strategically promote priming and boosting of local and systemic immune memory responses at times that match the greatest likelihood of BRSV exposure.

Acknowledgments

Funding for this study was obtained from the Alabama Agricultural Experiment Station–College of Agriculture, Auburn University.

The authors declare that there were no conflicts of interest. Presented as an oral abstract presentation at the Conference of Research Workers in Animal Disease, December 3, 2020.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org