Intranasal booster vaccination of beef steers reduces clinical signs following experimental coinfection with BRSV and BHV-1 without reducing shedding of BRD-associated bacteria

David A. Martínez, DVM, MS1; Manuel F. Chamorro, DVM, PhD, DACVIM1*; Thomas Passler, DVM, PhD, DACVIM1; Laura Huber, DVM, PhD, DACVPM2; Shollie Falkenberg, PhD2; Paul H. Walz, DVM, PhD, DACVIM2; Merrilee Thoresen, PhD1; Gage Raithel, BS2; Scott Silvis, MS2; Kiril M. Dimitrov, DVM, PhD4; Ricardo Stockler, DVM, PhD, DABVP1; Amelia R. Woolums, DVM, PhD, DACVIM, DACVM3

1Department of Clinical Sciences, College of Veterinary Medicine, Louisiana State University, Baton Rouge, LA
2Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL
3Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS
4Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX

*Corresponding author: Dr. Chamorro (mfc0003@auburn.edu)

OBJECTIVE
To determine the efficacy of primary or booster intranasal vaccination of beef steers on clinical protection and pathogen detection following simultaneous challenge with bovine respiratory syncytial virus and bovine herpes virus 1.

METHODS
30 beef steers were randomly allocated to 3 different treatment groups starting at 2 months of age. Group A (n = 10) was administered a single dose of a parenteral modified-live vaccine and was moved to a separate pasture. Groups B (n = 10) and C (10) remained unvaccinated. At 6 months of age, all steers were weaned and transported. Subsequently, groups A and B received a single dose of an intranasal modified-live vaccine while group C remained unvaccinated. Group C was housed separately until challenge. Two days following vaccination, all steers were challenged with bovine respiratory syncytial virus and bovine herpes virus 1 and housed in a single pen. Clinical and antibody response outcomes and the presence of nasal pathogens were evaluated.

RESULTS
The odds of clinical disease were lower in group A compared with group C on day 7 postchallenge; however, antibody responses and pathogen detection were not significantly different between groups before and following viral challenge. All calves remained negative for Histophilus somni and Mycoplasma bovis; however, significantly greater loads of Mannheimia haemolytica and Pasteurella multocida were detected on day 7 postchallenge compared with day −2 prechallenge.

CLINICAL RELEVANCE
Intranasal booster vaccination of beef steers at 6 months of age reduced clinical disease early after viral challenge. Weaning, transport, and viral infection promoted increased detection rates of M. haemolytica and P. multocida regardless of vaccination status.

Keywords: vaccination, bacteria, BRSV, BHV-1, antibody

Bovine respiratory disease (BRD) continues to cause major economic losses for the beef cattle industry in the US1,2 and is recognized as the leading cause of death of beef calves.3,4 Bovine respiratory syncytial virus (BRSV) is one of the most prevalent respiratory viruses in US cattle and plays an important role in the pathogenesis of BRD.5 Coinfections and synergism between viral and bacterial respiratory agents are characteristic of BRD and, in many cases, determine disease severity and clinical outcome. In 1 study,6 BRSV infection before challenge
with *Histophilus somni* led to greater levels of immunoglobulin E in bronchoalveolar fluid, more severe clinical disease, and greater lung pathology in calves, compared with calves challenged with BRSV or *H somni* alone. Additionally, results from a recent study demonstrated greater detection rates of *H somni* in nasal secretion samples of high-risk beef calves vaccinated with an intranasal (IN) modified-live vaccine (MLV) BRSV vaccine during the first 28 days following feedlot arrival compared with parenterally MLV-BRSV-vaccinated and control calves.

Bovine herpes virus 1 (BHV-1) is another important virus contributing to BRD that promotes secondary bacterial pneumonia in affected calves. Simultaneous exposure to multiple respiratory viruses including BRSV and BHV-1 may occur in highly commingled weaned beef calves marketed through auction barns and transported to stocker or feedlot operations. Although, fewer than 40% of US cow-calf producers vaccinate calves against respiratory viruses before weaning, the majority (>95%) of stocker and feedlot producers vaccinate newly acquired calves against respiratory viruses at arrival. IN MLV respiratory vaccines induce specific mucosal immunity (immunoglobulin A [IgA]) that provides local protection against infection with respiratory viruses in cattle; however, inconsistent clinical efficacy has been reported in young calves due to the short duration of mucosal immunity. IN MLV vaccination of beef calves around the time of arrival to stocker or feedlot operations could induce short-lived but effective immunity against exposure to multiple respiratory viruses. Results from a recent study demonstrated that a booster with an IN or parenteral MLV vaccination around weaning reduced clinical disease in calves challenged with BHV-1 4 days following the booster.

The first objective of this study was to determine if IN MLV vaccination of weaned beef calves following transport, either used as an initial vaccination or as a booster of the primary parenteral vaccination, results in differences in immune responses or provides clinical advantages compared with no vaccination against simultaneous experimental challenge with BRSV and BHV-1. The second objective was to compare the detection rate of bacterial respiratory pathogens in nasal secretion samples following transport, before vaccination, and after simultaneous experimental challenges with BRSV and BHV-1.

### Methods

#### Experimental design

The Auburn University Institutional Animal Care and Use Committee (PRN No. 2021-3970) reviewed and approved all animal protocols in this study. Thirty crossbreed beef steers from a single farm (Upper Coastal Plains Research Unit, Auburn University, Winfield, AL) were randomly assigned to 1 of 3 treatment groups at approximately 2 months of age. Group A (n = 10) received a single SC dose (2 mL) of an MLV vaccine (BOVILIS VISTA 5 SQ; Merck & Co, Inc) containing BHV-1, bovine virus diarrhea virus (BVDV) types 1 and 2, BRSV, and parainfluenza 3 virus. Groups B (n = 10) and C (10) received 2 mL of 0.9% saline, SC (Veltivex; Dechra Veterinary Products). Following treatment administration, group A was housed separately from groups B and C in a different pasture until weaning to prevent inadvertent vaccine-virus transmission (Table 1). At approximately 6 months of age (day −3), calves were abruptly weaned and transported (200 miles) as a single group to the North Auburn BVDV Research Unit (Auburn University), where all calves were housed together in a single pen. The next day (day −2), all calves were separated in individual holding pens by group in a single processing facility and chute system. Groups A and B were processed first and received a single dose (2 mL) of an IN MLV vaccine (Nasalgen 3; Merck & Co, Inc) containing BHV-1, BRSV, and parainfluenza 3 virus. Group C was processed last and received 2 mL of IN saline (Veltivex; Dechra Veterinary Products) and remained as the unvaccinated control group. Following treatment administration, groups A and B were placed in a single pen, and group C was placed in a separate pen to prevent inadvertent transmission of vaccine or viruses before challenge. On day 0, 15 calves (group A = 5, group B = 5, and group C = 5) were challenged with BRSV, and the other 15 were challenged with BHV-1. On the following day (day 1), calves were IN inoculated with the opposite virus with respect to day 0. Following day 0, calves from all groups were housed in a single pen. Clinical scores were recorded for all calves from day −2 to day 28. Serum samples were collected for serum neutralization analysis. Nasal secretion samples were collected for evaluation of local antibody responses and detection of BRSV, BHV-1, *H somni*, BVDV, and *Histophilus somni*.

### Table 1—Timeline, study procedures, and housing.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Procedures</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-months of age (branding)</td>
<td>Group A: MLV parenteral vaccination</td>
<td>Group A housed in separate pasture</td>
</tr>
<tr>
<td>Approximately 6 months of age, weaning (day −3)</td>
<td>Groups B and C: 0.9% parenteral saline</td>
<td>All groups housed in a single pen</td>
</tr>
<tr>
<td>Day −2</td>
<td>Abrupt weaning and transport to research unit</td>
<td>Groups A and B housed in a single pen</td>
</tr>
<tr>
<td>Days 0 to 1</td>
<td>Groups A and B: IN MLV vaccination. Group C: 0.9% IN saline</td>
<td>Group C housed in a separate pen</td>
</tr>
<tr>
<td>Days 5, 7, 10, 14, 21, and 28</td>
<td>Simultaneous challenge BRSV and BHV-1</td>
<td>All groups housed in a single pen</td>
</tr>
<tr>
<td>Postchallenge clinical evaluation and sampling</td>
<td>Clinical evaluation and sampling while each calf goes through the chute</td>
<td></td>
</tr>
</tbody>
</table>
**Mannheimia haemolytica, Pasteurella multocida, and Mycoplasma bovis.**

**BHV-1 and BRSV challenge**

For BHV-1 inoculation, calves were placed in a squeeze chute and administered 2 mL of an inoculum containing 3 x 10^5 cell culture infectious dose at 50% (CCID50) of BHV-1/mL (Colorado strain) in minimal Eagle medium IN using a nasal atomizer (MAD300 Nasal Intranasal Mucosal Atomization Device; Medline Industries LR). For the BRSV challenge, calves were placed in a sealed stock trailer measuring approximately 7.6 x 2.4 x 2.4 m with approximately 29 m^3 of air space. A lung wash inoculum of BRSV strain GA-1 with at least 1 cell culture passage and containing 1 x 10^5 CCID50 BRSV/mL was delivered to all calves via 2 ultrasonic nebulizers (SU99 Elite High-Flow Ultrasonic Induction Device/Nebulizer; WestPrime Healthcare; WestPrime Inc) placed on the opposite sides of the trailer at approximately 1.8 m above the trailer’s floor. Each ultrasonic nebulizer was loaded with 52.5 mL (approx 7 mL per calf) of inoculum nebulized for approximately 45 minutes.

**Clinical scoring and sampling**

Before weaning and transport, all calves (groups B and C) cohoused in a single pasture and group A in a different pasture) were observed by on-farm personnel not blinded to treatment allocation, who recorded daily clinical signs of disease (eg, nasal discharge, cough, depression, etc). A single veterinarian blinded to treatment allocation performed clinical scoring of calves before sampling once each individual calf was in the restraining chute on days −2, 0, 5, 7, 10, 14, 21, and 28. For clinical scoring, signs including depression, rectal temperature, respiratory rate, cough, nasal discharge, nasal erosions, and ocular discharge were evaluated on sampling days and a sum clinical score was assigned to each calf using a modification of a previously described respiratory scoring system (Supplementary Material S1).

Clinical scores were assigned in a scale of 0 to 3, where 0 was considered absent of abnormalities and 3 was the most abnormal clinical finding. The sum of individual scores including depression, rectal temperature, respiratory rate, cough, nasal discharge, nasal erosions, and ocular discharge determined the presence of mild or moderate to severe respiratory disease. Individual body weights were recorded on days −2, 14, and 28 using an electronic portable livestock scale (Livestock Platform Scale Brecknell) that was zeroed before weighing each animal.

Serum samples were collected at 2 months of age and on days −2 and 28 of the study by jugular venipuncture for serum neutralization assays. Nasal secretion samples to determine the presence of BRSV, BHV-1, *H. somni*, *M. haemolytica*, *P. multocida*, and *M. bovis* by reverse transcriptase quantitative PCR (RT-qPCR) and IgA titers for BRSV and BHV-1 by ELISA were collected on days −2, 5, 7, 14, 21, and 28. Nasal secretion samples were collected using cotton swabs attached to a 5-cm piece of nonabsorbable suture. The swabs were soaked in sterile 0.9% saline before being inserted in the calf’s left nostril for 3 minutes. After removal from the nostril, swabs were placed in 35-mL syringes, and secretions were expressed into plastic conical tubes. Samples from each calf were labeled such that treatment allocation remained blinded from personnel processing samples and performing laboratory assays.

**Virus neutralization**

Serum neutralization for BRSV and BHV-1 was performed as previously described. Serum samples were heat inactivated in a water bath at 55°C for 30 minutes, and then serial 2-fold dilutions from 1:2 to 1:1,024 were performed in 96-microwell flat-bottom plates, and 50 µL of media containing 100 to 500 CCID50 of virus (GA-1 strain for BRSV and Colorado strain for BHV-1) were added to wells. For each dilution, equal volumes of virus culture media were added to 3 wells. After dilution, the plates were incubated at 37°C in 5% CO2 for 1 hour, and Madin-Darby bovine kidney cells suspended in minimal Eagle medium (7% bovine serum and a solution containing streptomycin, penicillin, and amphotericin B) were added. The plates were incubated for 3 days and monitored daily for the presence of cytopathic effect by microscopic evaluation. Antibody titers were reported as the reciprocal of the lowest dilution of serum required to inhibit all cytopathic effects and results were log2 transformed for statistical analysis.

**ELISA for determination of anti-BRSV and anti-BHV-1 IgA titers in nasal secretions**

ELISA for determination of IgA in nasal secretions was performed as previously described. Briefly, viral particles of BRSV and BHV-1 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 for each virus was used to coat different 96-microwell polystyrene plates. After coating, the plates for each virus were incubated overnight at 4°C and washed 3 times with PBS containing 0.05% polysorbate 20 (Twee 20; Sigma-Aldrich). After being washed, 200 µL of PBSS solution containing 5% sheep serum albumin (Sigma-Aldrich) was added to each well for blocking, and the plates were incubated at 37°C for 1 hour and then washed 3 times. Nasal secretion samples were initially diluted 1:1 in Pluronic F127 and then diluted 1:100 in polysorbate 20. From this dilution, serial 2-fold dilutions were prepared from 1:25 up to 1:1,600 and each dilution was analyzed in triplicate. For virus-specific IgA, horseradish peroxidase-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]-diammonium salt substrate solution (Sigma-Aldrich) 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 anti-BHV-1 IgA yielded a green product when the bound peroxidase-conjugated rabbit anti-bovine IgA reacted with the substrates. IgA
RT-qPCR for BRSV

RT-qPCR was performed in nasal secretion samples as previously described. Briefly, RNA extraction from nasal secretion samples was performed using a commercially available reagent (RNAzol; Sigma-Aldrich). Once extracted, the RNA templates were reverse transcribed and amplified with qScript XLT One-Step RT-qPCR ToughMix (Quantabio) using BRSV-specific primers and probes. Each reaction (2.5 µL) was performed in a Bio-Rad CFX96 System and results were analyzed by the Bio-Rad CFX software manager. The detection limit of the assay was established at 10^3 BRSV RNA copies/µL. Samples were considered “detected” for BRSV if cycle threshold (Ct) values were below 35.

qPCR for BHV-1

A membrane kit (QiAamp MinElute Virus Spin Kit; Qiagen), was used to extract viral DNA from nasal secretion samples. A SYBR Green real-time PCR protocol was developed to detect BHV-1 as previously described. The reaction mixture contained 5 µL of DNA template, 10 µL of Advanced Universal SYBR Green Supermix (0.5 µM [0.5 µL]), and forward and reverse primers for BHV-1. All the reactions were performed in a qPCR detection system (Bio-Rad CFX96 System), and results were analyzed by the Bio-Rad CFX software manager. The detection limit of the assay was established at 10^3 BHV-1 DNA copies/µL. Samples were considered “detected” for BHV-1 if Ct values were below 35.

qPCR for bacteria

Total DNA was extracted from nasal secretion samples using the MagMAX-96 Viral RNA Isolation Kit AM1836 (ThermoFisher Scientific) and purified using Program AM1836-DW-One. Each sample was tested for H. somnii, M. haemolytica, P. multocida, and M. bovis by multiplex quantitative PCR immediately after DNA purification. Quantitative PCR was carried out using the Path-ID Multiplex One Step RT-qPCR kit (Life Technologies) for the multiplex reactions (M. haemolytica, P. multocida, and H. somnii) and the Path-ID qPCR Master Mix for the M. bovis reaction. For the multiplex reactions, the PCR mix contained 1 µL of nuclease-free water, 12.5 µL of 2X MP RT-PCR buffer, 1.0 µL PPM, 2.5 µL of 10X MP enzyme mix, and 8 µL of purified DNA. For the M. bovis reaction, the PCR mix contained 3.5 µL of nuclease-free water, 12.5 µL of 2X qPCR Master Mix, 1.0 µL of PPM, and 8 µL of purified DNA. The cycling conditions for all reactions were as follows: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 45 seconds with data collection at the second amplification step. Previously published sequences of primers and probes were used for PCR reactions including XIPC, M. bovis, M. haemolytica, and H. somnii. All quantitative PCR reactions were carried out on an ABI7500 Fast Real-Time PCR System (ThermoFisher Scientific) using standard plastic ware. Negative extraction control, no template control, and positive amplification control were utilized. Samples were considered “detected” for the targeted pathogens if Ct values were below 36.

Statistical analysis

Data were analyzed using statistical software (Rstudio, version 1.4.1717; The R Foundation). The normality of the data was assessed using the Shapiro-Wilk test and examination of the residuals. Generalized mixed-effects and mixed-effects logistic regression models were used to evaluate the effect of vaccination status and experiment time-point on the severity of clinical signs (a sum of clinical scores of ≤ 7 was determined mild, and a sum between 8 and 15 was determined moderate to severe). Body weight, BRSV and BHV-1 antibody titers in serum and nasal secretion samples, and detection of BRSV, BHV-1, and bacteria in nasal secretions. We used animal ID as the random effect to account for repeated measurements. Model selection was performed using stepwise regression, and only the best-fitted models, selected by comparing Akaike information criteria and the likelihood ratio test, were reported. If an interaction was detected, the interaction a description of its statistical significance was included. Post hoc familywise comparisons were performed using Tukey-Kramer with Bonferroni correction. Kaplan-Meier curves were generated to display BRSV, BHV-1, and bacteria detection in nasal secretions over time for calves in groups A, B, and C. Line graphs for categorical variables (for example, detection of BRSV, BHV, and bacteria by PCR techniques) were constructed by calculating the proportion of positives from all samples tested. For all analyses, significance was set at P ≤ .05.

Results

Clinical scores and average daily gain

Adverse reactions to vaccination or clinical signs of disease were not observed before viral challenge. Mortality was not observed during the entire study period. Following challenge with BRSV and BHV-1, signs of respiratory disease including fever (rectal temperature > 39.4°C), tachypnea, cough, nasal discharge, nasal erosions, and ocular discharge were moderate. The odds of developing severe clinical signs were 0.25 times as likely in group A compared with the control group (group C), and this association was statistically significant (95% CI, 0.07 to 0.96; P = .043; Table 2). Animals of all groups were 2.45 times as likely to develop severe clinical signs from day 10 to day 28 of the experiment compared with day −2 to day 7, and this association was statistically significant (95% CI, 1.03 to 5.84; P = .042; Table 2). The interaction between time point and group was not statistically significant (P = .08). The interaction
between the time point and group was not statistically significant and not included in the final model.

**BRSV- and BHV-1-neutralizing antibodies in serum**

Moderate mean ± SEM log₂ BRSV serum-neutralizing antibody (SNA) titers were present in all treatment groups at 2 months of age (A = 5.4 ± 0.5 vs B = 4.3 ± 0.4 vs C = 5 ± 0.5). In contrast, at the same time point, low mean ± SEM log₂ BHV-1 SNA titers were detected (A = 1.7 ± 0.6 vs B = 1.4 ± 0.6 vs C = 0.5 ± 0.3). A statistically significant effect of time (P < .05) was observed in the mean log₂ BRSV SNA titers between 2 months of age and day −2 and in BRSV and BHV-1 SNA titers between day −2 and day 28. In all treatment groups, BRSV SNA decayed by day −2 compared with titers present at 2 months of age; however, BRSV and BHV-1 SNA titers increased by day 28 following vaccination and viral challenge (Figure 1). Statistically significant differences in mean log₂ BRSV and BHV-1 SNA between treatment groups were not detected at any time point during the experiment. The interaction between time point and group was not statistically significant and not included in the final model.

**BRSV-IgA and BHV-IgA titers in nasal secretions**

Low mean ± SEM titers of nasal IgA for BRSV and BHV-1 were detected in calves from all treatment groups on day −2. The mean ± SEM nasal IgA titers for BRSV and BHV-1 increased in all treatment groups from day −2 to day 28 after challenge (Figure 2). An apparent greater increase in the mean ± SEM nasal IgA titers for BRSV and BHV-1 was observed in vaccinated calves (groups A and B) compared with controls (group C) on day 7; however, a statistically significant effect of time or treatment was not detected at any time point during the study period (P > .05). The interaction between time point and group was not statistically significant and not included in the final model.

**Detection of genetic material of BRSV and BHV-1 in nasal secretions by PCR assays**

On day −2, 20% of calves in each treatment group were positive for BRSV. At the same time point, 80%, 70%, and 70% of calves from groups A, B, and C, respectively, were positive for BHV-1. At every sampling point starting at day −2, a lower proportion of

---

**Table 2**—Association of time point and group with severity of clinical signs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (reference value = group C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>0.25</td>
<td>0.07–0.96</td>
<td>.043*</td>
</tr>
<tr>
<td>Group B</td>
<td>0.8</td>
<td>0.28–2.33</td>
<td>.686</td>
</tr>
<tr>
<td>Time point (reference value: day −2 to day 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 10 to day 28</td>
<td>2.45</td>
<td>1.03–5.84</td>
<td>.042*</td>
</tr>
</tbody>
</table>

OR, 95% CI, and P values derived from logistic regression with severity of clinical signs (mild vs moderate to severe) as main outcome and time point (day −2 to day 7 vs day 10 to day 28) of the experiment and group as the main response variables.

*P ≤ .05, statistical significance.

---

**Figure 1**—Mean ± SEM bovine respiratory syncytial virus (BRSV; A) and bovine herpes virus 1 (BHV-1; B) serum-neutralizing antibody titers for calves in vaccinated groups (groups A and B) and the control group (group C) at 2 months of age (2mo), 2 days prior viral challenge (D−2), and day 28 (D28) of the experiment. Data were analyzed using generalized mixed-effects models. Different letters (a and b) represent significant (P < .05) difference between time points within participants of each group. Familywise multicomparisons were performed using Tukey-Kramer with Bonferroni correction. Statistically significant differences between groups were not observed at any time point.
calves were positive to BRSV compared with the proportion of calves positive to BHV-1 across all treatment groups (Figure 3). The proportion of calves positive for BRSV was low and did not significantly vary from days −2 to 28. In contrast, a significantly lower (P < .05) proportion of calves were positive for BHV-1 on days 21 and 28 of the study compared with days −2, 5, 7, and 10; however, a statistically significant effect of treatment was not detected for any of the viruses at any time point.
Based on the detection of viruses by PCR assays, between days −2 and 28, the risk of shedding BRSV and BHV-1 was not significantly different across time (P = .6 and 1, respectively; Figure 4), and statistically significant differences were not detected among treatment groups at any time point. Moreover, the interaction between time point and group was not statistically significant and not included in the final model.

**Figure 4**—Kaplan-Meier curves of the cumulative probability of shedding bovine respiratory syncytial virus (BRSV; A) and bovine herpes virus 1 (BHV-1; B) detected with reverse transcriptase-PCR and PCR, respectively, for calves in vaccinated groups (group A, red; group B, purple) and control group (group C, gray) on time intervals 0 to 7, in which each time interval is representing days −2 to 0, 0 to 5, 5 to 7, 7 to 10, 10 to 14, 14 to 21, and 21 to 28 following viral challenge. In each graphic, tick marks represent the end of each time, each step represents detection events of BRSV and BHV-1 shedding, and shading represents the respective 95% CI for the probability of shedding BRSV and BHV-1 by calves.

**Figure 5**—Mean proportion ± SEM for *Pasteurella multocida* (A) and *Mannheimia haemolytica* (B) detection for calves in vaccinated groups (groups A and B) and the control group (group C) on days −2, 5, 7, 14, and 28 of the experiment. Data were analyzed using mixed-effects logistic regression. Different letters (a and b) represent significant (P < .05) difference between time points within participants of each group. Familywise multicomparisons were performed using Tukey-Kramer with Bonferroni correction. Statistically significant differences between treatment groups were not observed at any time point.
Detection of genetic material of *H somni*, *M haemolytica*, *P multocida*, and *M bovis* in nasal secretions by PCR

*Histophilus somni* and *M bovis* were not detected in any calf from any treatment group at any time point. On day −2, *M haemolytica* was not detected in calves in group A, and *M haemolytica* was minimally detected (Ct range > 35 ≤ 45) in 11% of calves from groups B and C, respectively. In contrast, on day −2 *P multocida* was detected in 63%, 78%, and 55% of calves of groups A, B, and C, respectively. Following day −2, the rate of detection of *P multocida* and *M haemolytica* increased in all groups until day 7 (Figure 5). The odds of *P multocida* detection were significantly greater (*P* < .05) on day 7 compared with days 14 and 28. Similarly, the odds of *M haemolytica* detection were significantly greater (*P* < .05) on day 7 compared with day 28 across all treatment groups. Statistically significant differences between treatment groups were not observed at any time point. Moreover, the interaction between time point and group was not statistically significant and not included in the final model.

**Discussion**

In this study, our goal was to replicate conditions typical of abruptly weaned and transported beef calves entering stocker/backgrounder operations. Administering an IN MLV vaccine to beef calves of unknown health or vaccination status at arrival to stocker or feedlot operations, although not commonly practiced, could become an alternative to stocker or feedlot operations, although not commonly practiced, could become an alternative to prime local mucosal immunity and reduce mortality due to BRD during the first weeks after arrival. Results from a previous study demonstrated that primary vaccination or booster (4.5 months after primary vaccination) with an IN MLV vaccine at weaning reduced mortality due to *M haemolytica* pneumonia in beef calves challenged with BHV-1 4 days following IN MLV vaccination. Additionally, in the same study, a combination vaccine protocol with an IN MLV vaccine at 3 to 6 weeks of age and an IN MLV booster at 6 months of age resulted in a significant reduction of clinical disease. In this study, the odds of developing severe clinical signs were significantly lower in group A calves compared with control calves (group C). Moreover, the odds of developing severe clinical signs increased significantly in all groups on days 10 to 28 compared with days −2 to 7. Numerically greater nasal IgA titers against BRSV and BHV-1 present in vaccinated calves compared with controls on the same day could have contributed to lessening clinical disease in these groups; however, clinical outcomes and local immune responses were not significantly different between treatment groups. It is possible that lack of sufficient statistical power resulting from the small sample size prevented detection of significant differences. Alternatively, it is possible that innate or adaptive local immune responses induced by IN MLV vaccination or natural infection with BRSV and BHV-1 before challenge influenced the clinical outcomes observed in this study. These findings are consistent with results from previous studies that demonstrated reduced mortality in calves vaccinated with a combination vaccine protocol or primary MLV vaccination before BHV-1 or BRSV challenge.

The presence of low nasal IgA titers to BHV-1 and BRSV and positive PCR results to both viruses on day −2, before IN vaccination and viral challenge, suggest previous natural exposure and infection. This is not entirely surprising given the high seroprevalence and endemic nature of these viruses in cattle operations from the US. It is possible that the stress of abrupt weaning and transport contributed to viral shedding and transmission between calves from different treatment groups. Results from previous studies suggested that shedding and transmission of BRSV and BHV-1 from chronically or latently infected cattle can occur in endemic farms where BRSV and BHV-1 circulate amongst cattle populations. Additionally, latent infection with BHV-1 has been reported in cattle previously vaccinated with MLV BHV-1-containing vaccines, and BRSV shedding occurs in latently infected cattle following stressful events or immunosuppressive treatment. It is possible that calves from group A that were vaccinated with a parenteral MLV BHV-1 at 2 months of age developed a latent BHV-1 infection and shed the virus following weaning and transport.

Primary IN MLV vaccination or IN MLV booster at 6 months of age, 2 days before experimental challenge, and in the face of natural viral exposure did not result in a significant reduction of BRV and BHV-1 detection in nasal secretions; however, a significantly lower proportion of calves were positive for BHV-1 on days 21 and 28. Depending on the immune status of the animal, natural exposure or IN MLV vaccination against BRSV and BHV-1 can take between 6 and 35 days to induce mucosal immune responses that prevent subsequent viral infection. The natural course of BHV-1 infection and the progressively increasing nasal IgA titers after challenge could have reduced viral replication and detection on days 21 and 28. Results from previous studies demonstrated a reduction of BRSV and BHV-1 nasal shedding following challenge of calves vaccinated as close as 4 days prior with combination vaccine protocols including IN vaccines. Natural exposure to BHV-1 and BRSV and IN MLV vaccination thereafter likely influenced the nasal detection of these viruses following experimental challenge in this study. Results from a recent study demonstrated a 100% positive rate for BHV-1 and BRSV by PCR between 3 and 21 days postvaccination in calves vaccinated with IN MLV vaccines.

Compared with BRSV and despite the presence of similarly increasing levels of nasal IgA for both viruses following IN vaccination and challenge, a greater proportion of calves were positive for BHV-1. This could be associated with the higher titer of inoculated BHV-1 compared with BRSV in our challenge model or an ineffective BRSV model in our study. It is possible that the passage of the BRSV inoculum...
through cell cultures before challenge reduced viral virulence, fitness, and ability to replicate and disseminate through the respiratory tract. Alternatively, BHV-1 has a strong tropism for the upper respiratory tract of cattle and its ability to replicate and move transcellularly across epithelial cells could have negatively affected BRSV replication and detection. Additionally, results from previous studies demonstrated a reduced probability of detecting BRSV by RT-PCR in nasal secretions of calves with BRD compared with transtracheal wash or bronchoalveolar fluid samples. Unfortunately, we did not collect lower respiratory fluid samples to compare the rate of detection of both viruses in each anatomical location.

Regardless of vaccination status, SNA titers to BRSV and BHV-1 decayed from 2 months to 6 months of age (day −2) across all treatment groups. This reflects maternal interference with vaccination in calves from group A at 2 months of age. Following IN vaccination and challenge, SNA to both viruses increased similarly in all treatment groups. Previous studies demonstrated similar SNA titers for BRSV and BHV-1 in 6-month-old beef calves previously vaccinated with a combination MLV vaccine protocol at birth and 2 months of age or at 2 months of age and 6 months of age. Similar levels of nasal IgA titers for BRSV and BHV-1 were present in calves from all groups on day −2; however, vaccinated calves (groups A and B) had greater nasal IgA titers to BRSV and BHV-1 on day 7 compared with control calves (group C). Although not statistically significant, the difference in the nasal IgA response on day 7 following experimental viral infection in vaccinates versus controls may indicate advantages of IN MLV vaccination at arrival of calves exposed to respiratory viruses by the time of arrival at stocker or feedlot operations.

In this study, the detection rate of M haemolytica and P multocida in nasal secretion samples from calves across treatment groups increased on day 7 compared with day −2 (IN MLV vaccination) and following experimentally infection with BRSV and BHV-1 (day 0). It is possible that the presence of M haemolytica and P multocida in nasal secretions was a reflection of their presence in the lower respiratory tract. A high level of agreement between the detection of M haemolytica, P multocida, and M bovis in nasal secretion and transtracheal wash samples from calves with clinical BRD was previously reported. Results from 1 study demonstrated that a transportation event increases the detection of M haemolytica, P multocida, and M bovis in nasal secretion samples of beef steers following arrival to the feedlot. Additionally, results from a recent study demonstrated an increased rate of detection of H somni during the first 28 days following feedlot arrival in nasal secretion samples from multisourced high risk beef calves from Texas previously vaccinated with an IN MLV vaccine. The authors of that study suggested that the BRSV component of the IN MLV vaccine could have altered the nasal microbiota of vaccinated calves favoring the colonization and growth of H somni. In contrast, calves from this study remained negative for H somni and M bovis in nasal secretion samples after transport and following IN MLV vaccination and experimental challenge with BRSV and BHV-1. It is possible that other factors different from IN MLV vaccination, such as commingling and geographic location have, an effect on the presence and growth of H somni in the upper respiratory tract of cattle.

Important limitations of this study include the small sample size, the inadvertent natural exposure of study calves to BRSV and BHV-1 before challenge, and the use of single-sourced calves to replicate conditions typical of weaned beef calves entering stocker or feedlot operations. It is possible that the small sample size reduced the power necessary to demonstrate significant differences in clinical outcomes and immune responses following experimental challenge of calves. The inadvertent natural exposure to BRSV and BHV-1 before challenge prevented the evaluation of vaccination efficacy due to the lack of a true naïve control group. Multisourced highly commingled calves would have been ideal to represent conditions typical of stocker cattle. However, despite its limitations, the results from this study provide valuable information on upper respiratory immune responses and bacterial predominance of beef calves exposed to BRSV and BHV-1 by the time of weaning, transport, and arrival to their next stage of production.

Natural exposure of study calves to BRSV and BHV-1 before IN MLV vaccination and challenge resulted in circumstances similar to those experienced by weaned beef calves arriving at stocker or feedlot operations in the US. Under these circumstances and based on the results of this study, IN MLV vaccination at arrival could be an alternative to improve clinical protection by boosting timely upper respiratory IgA-specific responses.

Acknowledgments

None reported.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

Funding

Funding was provided by the Alabama Agricultural Experiment Station, and by the Department of Clinical Sciences, College of Veterinary Medicine, Auburn University.

References


**Supplementary Materials**

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