INTRODUCTION

Bovine herpesvirus 1 (BHV-1) is associated with several disease syndromes in cattle. BHV-1 respiratory infections can result in several clinical manifestations ranging from subclinical to severe infectious bovine rhinotracheitis (IBR).\(^1\) BHV-1 infection in calves and feedlot cattle can lead to bovine respiratory disease complex and secondary bacterial infections that cause significant economic losses to the livestock industry. Clinical signs include pyrexia, anorexia, conjunctivitis with lacrimal discharge, dyspnea, and nasal discharge.\(^2\) BHV-1 infections can also result in infectious pustular vulvovaginitis\(^3\) and abortions\(^4\) in adult animals.

Vaccination programs are a routine practice in US feedlot operations to protect cattle against disease associated with BHV-1.\(^5\) Numerous commercial vaccines for the prevention of IBR-related respiratory diseases have been available since the 1950s.\(^6\) Current vaccine protocols recommend vaccination of calves before weaning or commingling to provide maximum protection against IBR. Unfortunately, many calves are not vaccinated according to these recommendations, placing them at risk...
The time from vaccination to the onset of protection can play an important role in subsequent management of newly arrived cattle in the feedlot. Previous studies have demonstrated that calves vaccinated 24 to 96 hours before exposure are protected against clinical disease caused by IBR.\(^8\)\(^-\)\(^12\) Protection against IBR has occurred as early as 24 hours after vaccination with an intranasal (IN) vaccine. IN vaccinations have an advantage over parenteral vaccination in the provision of a shorter period from vaccination to stimulation of active immunity.

### TABLE 1. Incidence of Clinical Signs in Groups of Calves Vaccinated with a Commercial Modified-Live Virus Multivalent (Bovine Herpesvirus 1, Bovine Viral Diarrhea Virus 1 & 2, Parainfluenza-3, Respiratory Syncytial Virus) Vaccine at Various Intervals Before Intranasal Challenge with Bovine Herpesvirus 1

<table>
<thead>
<tr>
<th>Sign</th>
<th>Study Day</th>
<th>Vaccinated 96 hr Before Challenge</th>
<th>Vaccinated 72 hr Before Challenge</th>
<th>Vaccinated 48 hr Before Challenge</th>
<th>Nonvaccinated Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td></td>
<td>2 (18%)†</td>
<td>1 (9%)†</td>
<td>8 (73%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
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<td>4</td>
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<td></td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total*</td>
<td></td>
<td>6 (54%)</td>
<td>5 (45%)</td>
<td>9 (81%)</td>
<td>9 (90%)</td>
</tr>
</tbody>
</table>

*Total number of calves that exhibited given sign on one or more days.

†Significantly less than percentage for control group (\(P \leq .03\)).
However, IM vaccinations appear to have similar levels of protection and produce similar levels of both nasal and serum antibodies. Protection against BHV-1 challenge has occurred as early as 48 hours after IM vaccination.

A study was conducted to determine the onset of protection against an IN challenge with BHV-1 following a SC injection of a commercial modified-live virus (MLV) multivalent vaccine containing attenuated BHV-1.

**MATERIALS AND METHODS**

**Animals**

All animal procedures were approved by the Rural Technologies, Inc. Institutional Animal Care and Use Committee. Forty-three (33 vaccinates and 10 nonvaccinated controls) Limousin-cross steers, 6 to 8 months of age, weighing approximately 240 kg each, and with serum neutralization (SN) antibody titers less than 1:2 to IBR, were acquired for the study. Calves were weaned on the day of arrival (Day –16), and each received 10 mg tilmicosin/kg (Micotil, Elanco Animal Health) SC to prevent secondary bacterial pneumonia during the acclimation period. The calves were individually weighed, blocked by weight into three groups of 11 and one group of 10, and allocated within each weight block to four treatment groups by random drawing. Calves in three of the treatment groups (n = 11) were vaccinated 96 (Day –4), 72 (Day –3), or 48 (Day –2) hours before challenge. The final group (n = 10) consisted of nonvaccinated controls.

Animals were commingled throughout the study except between Days –4 and 0, when vaccinates were separated from controls. Animals were housed at a research farm near Brookings, SD, and were fed an age-appropriate diet; water was supplied ad libitum.

**Vaccine**

A commercial MLV vaccine (Express 5, Boehringer Ingelheim Vetmedica) containing BHV-1, bovine viral diarrhea virus 1 and 2, parainfluenza-3, and bovine respiratory syncytial virus was used for the study. Vaccinates received a single dose (2 ml) of reconstituted vaccine by SC injection in the neck.

**Challenge Virus**

The Cooper strain of BHV-1 was obtained from the National Veterinary Service Laboratory, Ames, IA, and stored at –80°C until use. For the challenge, 45 ampules of virus were rapidly thawed, pooled, and diluted with Eagle’s minimum essential medium to a final volume of 180 ml. Diluted virus was divided into 2-ml aliquots in snap-cap tubes, placed on ice, and transported to the challenge site. Animals received $5 \times 10^5$ tissue culture 50% infective dose (TCID$_{50}$/ml of virus on the day of challenge (Day 0).

**Experimental Challenge**

All animals were challenged on the same day (Day 0). Immediately before challenge, a 500-ml biohazard bag was placed over the muzzle of the calf to induce anoxia. When deep breathing was observed, the bag was removed and the virus administered using an aerosol unit (Chromist TLC atomizer; Gelman Sciences) by spraying the challenge virus into the back of the nares on inspiration. Four ml was administered to each animal (2 ml/naris). The biohazard bag was then placed over the muzzle again until deep breathing was observed.

**Clinical Assessments**

Personnel performing clinical assessments were blinded to the treatments. Animals were observed daily from Days –4 through 14 for vaccine reactions and clinical signs associated with IBR infection, including ocular discharge (lacrimation or conjunctivitis), respiratory character (rapid short breaths, mild or severe dyspnea with grunts), coughing, nasal dis-
charge (normal, mild, moderate, or heavy), nasal lesions, depression, and anorexia. Rectal temperatures for all animals were monitored daily from Days 0 through 14.

Calves were individually weighed on Days –1, 14, and 29 to evaluate differences in average daily gain (ADG; (final weight – starting weight)/(number of days in period).

Blood was collected via jugular venipuncture on Days 0 and 14 into serum separator tubes for determination of IBR SN antibodies at Iowa State University Veterinary Diagnostic Laboratory, Ames, IA, using a standard SN assay. A constant virus (200 TCID₅₀) was incubated with twofold serial dilutions (1:2–1:4096) of sera before inoculation of Madin-Darbin bovine kidney (MDBK) cells in microtiter tissue culture plates. Plates were incubated at 37°C with 5% carbon dioxide for 5 days before visual assessment of virus induced cytopathic effect.

Nasal secretions (0.5–1 ml) were collected by aspiration on Days –1, 3, and 6 and tested for virus isolation, using a standard virus isolation assay. Serial 10-fold dilutions of samples were made, and each diluted sample was added in triplicate to MDBK cell monolayers in microtiter tissue culture plates. Samples on culture plates were incubated for 5 days at 37°C with 5% carbon dioxide before being evaluated by cytopathic effect and IBR immunofluorescence staining. Samples were considered negative for IBR virus if no cytopathic effect or virus-specific fluorescence was observed in inoculated cells after one blind passage.

Statistical Analysis

PROC MIXED procedures of SAS (Version 8.2; SAS Institute) were used for all analyses of variance and covariance. The PROC FREQ procedure was used for all categoric data analyses. Differences were considered significant at $P \leq .05$.

Body weights were analyzed using a one-sided $t$-test; temperature, serology, and virus isolation were analyzed using an analysis of variance. In the presence of significant differences among groups, pairwise comparisons were made between the control group and each vaccinate group using a one-sided $t$-test with the alternative hypothesis that the mean for the treated group is greater than the mean for the control group. Pairwise comparisons using one-sided $t$-tests were made between individual vaccinate groups if the means of both of the treatments in the comparison were significantly greater than the mean for the control group.

Clinical signs were analyzed using the Cochran-Mantel-Haenszel test. In the presence of significant differences among groups, the same analysis was used. If the response variable had two categories, a one-sided test with an $\alpha$-level of 0.05 was accomplished by evaluating a two-sided test with an $\alpha$-level of 0.10, given that the percentage for the control group is greater than that of the treated group. If there were more than two categories in the response variable, a two-sided test was used.

RESULTS

Clinical Observations

Depression

Mild to moderate depression was observed on various days between Days 3 and 11 in two (18%) calves vaccinated 96 hours, one calf (9%) vaccinated 72 hours, and eight (73%) calves vaccinated 48 hours before challenge (Table 1). Depression was observed in six (60%) nonvaccinated calves. The percentage of animals vaccinated either 96 or 72 hours before challenge that became depressed was significantly ($P \leq .03$) less than for the nonvaccinated group. No differences were observed between the group vaccinated 48 hours before challenge and the nonvaccinated group.

Nasal Discharge

All animals from all treatment groups had an
increase in nasal discharge after challenge during the 14-day observation period (data not shown). Only on Day 10 was nasal discharge significantly \((P \leq .03)\) less for groups vaccinated either 96 or 72 hours before challenge than for nonvaccinated calves. There were no significant differences in nasal discharge scores between the group vaccinated 48 hours before challenge and the nonvaccinated controls.

**Ocular Lesions**

Nine of 10 (90%) nonvaccinated animals had moderate to severe lacrimation and conjunctivitis, whereas two (27%) animals vaccinated 96 hours \((P = .0007)\), five (45%) vaccinated 72 hours \((P = .05)\), and six (54%) vaccinated 48 hours before challenge exhibited similar clinical signs. These signs commenced on Day 2 and were resolved by Day 8 for groups vaccinated either 96 or 72 hours before challenge, whereas lesions were still present in two (20%) nonvaccinated calves on Day 14.

**Respiratory Character**

Changes in respiratory character for all groups began on Day 3. Abnormal respiration was seen on Days 3 through 8 in six (54%) calves vaccinated 96 hours \((P < .05\) during Days 8–11) and five (45%) animals vaccinated 72 hours \((P < .05\) during Days 8–11) before challenge (Table 1). Nine calves (81%) vaccinated 48 hours before challenge and nine (90%) nonvaccinated calves displayed abnormal respiration during Days 3 through 14 (Table 1). In addition, nonvaccinated controls as well as animals vaccinated 48 hours before challenge showed signs of severe dyspnea during the post-challenge observation period.
TABLE 2. Body Weight Data and Average Daily Gain (ADG) for Groups of Calves Vaccinated with a Commercial Modified-Live Virus Multivalent (Bovine Rhinotracheitis, Bovine Viral Diarrhea Virus 1 & 2, Parainfluenza-3, Respiratory Syncytial Virus) Vaccine at Various Intervals Before Intranasal Challenge with Bovine Herpesvirus 1

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day –1</th>
<th>Day 14</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Body Weight (kg)</td>
<td>Mean Body Weight (kg)</td>
<td>Total Gain (kg)</td>
</tr>
<tr>
<td>Vaccinated 96 hr before challenge</td>
<td>240.2</td>
<td>256.1</td>
<td>15.9</td>
</tr>
<tr>
<td>Vaccinated 72 hr before challenge</td>
<td>243.4</td>
<td>255.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Vaccinated 48 hr before challenge</td>
<td>235.3</td>
<td>244.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Nonvaccinated controls</td>
<td>242.2</td>
<td>246.2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*Calculated using the formula (Vaccinated group gain – Nonvaccinated group gain)/Nonvaccinated group gain × 100.
†Significantly greater than ADG for nonvaccinated group (P ≤ .04).
‡Significantly greater than ADG for nonvaccinated group (P ≤ .004).

Body Temperatures

All nonvaccinated calves had a body temperature reading of 40.6˚C (105˚F) or higher on one or more days after challenge, whereas groups vaccinated either 96 or 72 hours before challenge each had one animal and the group vaccinated 48 hours before challenge had three animals with temperatures 40.6˚C or greater on one or more days after challenge (Figure 1). Mean body temperature of calves vaccinated 96 or 72 hours before challenge never exceeded 40˚C (104.0˚F) during the 14 days after challenge; peak body temperatures occurred on Day 3 for calves vaccinated 96 hours before challenge (39.7˚C) and on Day 2 for calves vaccinated 72 hours before challenge (39.9˚C). Mean body temperature for animals vaccinated 48 hours before challenge reached or exceeded 40˚C on Days 3 and 4. The mean body temperature in the nonvaccinated calves peaked at 41˚C (105.8˚F) on Day 4; however, the mean for this group was 40˚C or below from Days 4 through 6. Mean body temperature in the nonvaccinated group was significantly (P ≤ .03) higher on Days 2 through 9 than that for the group vaccinated 96 hours before challenge, on Days 3 through 9 for calves vaccinated 72 hours before treatment, and on Days 4 through 6 for calves vaccinated 48 hours before challenge.

Body Weight

ADG for groups vaccinated either 96 or 72 hours before challenge was significantly (P ≤ .04) higher than for the nonvaccinated group.
on Day 14 (Figure 2, Table 2). ADG to Day 29 was significantly \((P \leq 0.04)\) higher only for the group vaccinated 96 hours before challenge. ADG for calves vaccinated 48 hours before challenge was statistically similar to that for nonvaccinated calves. Over the 29 days of the study, calves vaccinated 96 hours before challenge gained approximately 76% more weight than did nonvaccinated controls (Table 2).

### DISCUSSION

This study provided evidence that a single dose of a commercially available multivalent MLV vaccine containing attenuated BHV-1 administered SC to calves 72 or 96 hours before challenge with BHV-1 provided significantly \((P \leq 0.05)\) adequate protection against the development of clinical disease (IBR). Calves vaccinated according to either schedule had significantly \((P \leq 0.05)\) better weight gains for 14 to 29 days, diminished clinical signs, lower body temperatures, and reduced viral shedding.

The vaccinated calves in this study gained approximately 39% to 76% more weight than the nonvaccinated controls. Total gains achieved were less than those previously reported for calves vaccinated parenterally 48 hours or more before exposure\(^\text{11}\); however, vaccinated animals in the earlier study were challenged with BHV-1 via contact, whereas a more severe IN challenge was used in this study.

In the present study, calves vaccinated 72 or 96 hours before challenge exhibited mild or no clinical signs (depression, respiration, nasal discharge) after IN challenge when compared with nonvaccinated control calves. Results of
this study support findings of earlier studies in which parenteral vaccinations provided early protection against clinical signs of IBR.9–11 One of these studies11 reported that calves were protected against clinical signs as early as 48 hours after vaccination, using a contact challenge design. In another study,9 IN vaccination administered to calves less than 40 hours before IN challenge was not effective in reducing clinical signs associated with IBR. Others10 have reported a reduction in clinical signs 24 hours after IN vaccination in a study using a contact challenge method.

The mechanisms of early protection against challenge with BHV-1 are not fully understood. Studies have demonstrated that interferon-gamma (INFγ) may play a role in protection after challenge.14–16 Levels of INFγ were not monitored in this study; however, SN antibodies were not present in animals up to 4 days after vaccination, indicating that antibodies play a small role in disease protection soon after vaccination.

Based on the results of this study, it was concluded that SC administration of this commercially available MLV vaccine to nonvaccinated calves at weaning or at arrival to feed yards will provide significant (P < .05) weight gain benefits and decreased clinical signs and viral shedding as early as 72 hours before exposure to other animals with IBR when calves from different sources are commingled.

ACKNOWLEDGMENTS

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REFERENCES

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