ABSTRACT

The purpose of the study reported here was to compare four different vaccine regimens for their efficacy in protecting calves from challenge with bovine herpesvirus 1 (BHV1) at 5 and 14 days after vaccination. Nine experimental groups of five calves each were used to compare four vaccination regimens and a group that received no vaccine. The four vaccine regimens were: a BHV1 intramuscular (IM) vaccine containing both modified live virus (MLV) and killed virus (KV); an MLV intranasal (IN) vaccine; concurrent administration of MLV IN vaccine and MLV IM vaccine; and concurrent administration of MLV IN vaccine and KV vaccine. All vaccine regimens induced solid protection against BHV1 challenge at 14 days after vaccination. All of the vaccine regimens also induced statistically significant (P < .05) protective immunity at 5 days after vaccination; however, there were significant differences (P < .05) in the degree of protection. The best protection induced by 5 days after vaccination was provided by the MLV + KV combination vaccine injected IM and the MLV vaccines given IN and IM concurrently. The MLV + KV combination vaccine administered IM gave significantly (P < .05) better protection by 5 days after vaccination than the MLV vaccine administered IN. Administering a KV BHV1 vaccine IM at the same time as the MLV BHV1 IN vaccine significantly (P < .05) reduced the effectiveness of the IN vaccine for inducing early protective immunity.

INTRODUCTION

It is sometimes very important to induce rapid protection against infection with bovine herpesvirus 1 (BHV1) after vaccination. This is especially important when vaccines are used at arrival for feedlot cattle or when they are used early in an outbreak of infection with BHV1 in a herd.¹ Shortening the interval from vaccina-
tion to induction of protective immunity can significantly reduce the severity of disease in the herd under these circumstances. There are many types of vaccines licensed by the United States Department of Agriculture because they have been shown to be safe and effective for preventing BHV1-induced disease after challenge. Typically the challenge is administered 2 to 5 weeks after one or two doses of vaccine is administered. Intranasally administered modified live virus (MLV) vaccines have been shown to induce measurable protection against experimental challenge by 3 days after administration or against contact exposure by 48 hours after vaccination. In contrast, others have reported the failure of intranasally administered MLV vaccines to protect against challenge with BHV1 at 3 days after administration. Intramuscularly administered MLV vaccines have been shown to provide measurable protection against contact exposure to BHV1 by 2 days after vaccination. These studies focused on demonstrating significant protection of vaccinated animals compared with nonvaccinated animals. The study reported here focused on demonstrating significantly improved protection when various vaccination regimens were compared.

Veterinarians have recommended various vaccination protocols in an attempt to induce stronger immunity to BHV1 more rapidly. These protocols have included using various combinations of MLV vaccines given intramuscularly (IM) and/or intranasally (IN) with or without the concurrent use of killed virus (KV) vaccines. The purpose of the study reported here was to compare four vaccination regimens for their ability to induce protection from BHV1 challenge at 5 and 14 days after vaccination.

**MATERIALS AND METHODS**

**Experimental Design**

Forty-five calves (250 to 300 kg) seronegative for antibodies to BHV1 were individually identified using ear tattoos and tags and randomly assigned to nine groups of five calves each. Table 1 shows the groups, vaccines administered, route of administration, day of vaccination, and day of challenge.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Calves</th>
<th>Vaccine*</th>
<th>Route</th>
<th>Vaccination Day</th>
<th>Challenge Day</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>A</td>
<td>IM</td>
<td>0</td>
<td>14</td>
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<tr>
<td>2</td>
<td>5</td>
<td>A</td>
<td>IM</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>B</td>
<td>IN</td>
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<td>14</td>
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<tr>
<td>5</td>
<td>5</td>
<td>B&amp;C</td>
<td>IN/IM</td>
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<td>14</td>
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<tr>
<td>6</td>
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<td>IN/IM</td>
<td>9</td>
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<td>IN/IM</td>
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<td>B&amp;D</td>
<td>IN/IM</td>
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<td>14</td>
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<tr>
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<td>5</td>
<td>NONE</td>
<td>N/A</td>
<td>N/A</td>
<td>14</td>
</tr>
</tbody>
</table>

IM = Intramuscular
IN = Intranasal
*See Table 2 for description of vaccines.

![Table 1. Experimental Design](image-url)
tion of the vaccines is found in Table 2. The calves were treated with vitamin A and D, vitamin E-selenium, vitamin B complex, and ivermectin upon arrival. Decoquinate (Decox\textsuperscript{®}, Alpharma, Fort Lee, NJ) was placed in the feed for the duration of the study. The animals were rested and acclimated to their environment for 2 weeks before experimental procedures were started. From day –14 to day 0 of the study all calves were housed together. From day 0 to day 9 the calves in the nine treatment groups were housed in four separate pens as follows: pen 1 (group 1); pen 2 (group 9); pen 3 (groups 3, 5, and 7); pen 4 (groups 2, 4, 6, and 8). From days 9 to 24 the calves in the nine treatment groups were housed in four separate pens as follows: pen 1 (groups 1 and 2); pen 2 (group 9); pen 3 (groups 3, 5, and 7); pen 4 (groups 4, 6, and 8). All calves were challenged intranasally with the United States Department of Agriculture (USDA) recommended challenge dose of BHV1 ($10^{8.6}$ TCID\textsubscript{50} of the Cooper’s strain of BHV1; lot number 88-9) obtained from the USDA, Animal and Plant Health Inspection Service (APHIS), National Veterinary Services Laboratories (Ames, IA).

### Clinical Observations

Clinical signs evaluated included dyspnea, coughing, nasal discharge, and apathy. Each clinical sign was evaluated independently (on days 14 through 24) by two individuals who were unaware of the vaccines each animal received and who graded each clinical sign using the following scale: 0 (normal); 1 (slight); 2 (mild); 3 (moderate); or 4 (severe). The two scores for each clinical sign were averaged for each day. A composite clinical score for each animal for each day was calculated by adding the score for each of the clinical signs and the rectal temperature.

### BHV1 Titration in Nasal Secretions

Nasal secretions were collected by suction from each ventral nasal meatus through a modified pipette into a trapping tube.\textsuperscript{13} The nasal secretions were stored frozen at –20°C until tested as previously described.\textsuperscript{14} BHV1 in nasal secretions was quantified by thawing, sonicating, and centrifuging (15,600 $\times$ gravity for 3 minutes) the nasal secretions and inoculating two secondary bovine turbinate cell monolayers with 0.2 mL of ten-fold serial dilutions of the supernatant fluid. After 1 hour adsorption, the cells were overlaid with MEM 410-1500 (GIBCO Laboratories, Gaithersburg, MD)—containing 7.0 g granulated agar (DIFCO Laboratories, Detroit, MI), 30 mL heat-inacti-
activated fetal bovine serum, 100 mg gentamicin (Schering Corp, Kenilworth, NJ), and 50,000 units of mycostatin (E.R. Squibb and Sons, Inc, Princeton, NJ) per liter—and incubated at 37°C in a 5% CO₂ atmosphere for 5 days. The agar was removed, the cell layer was stained with crystal violet, and the viral plaques were enumerated.

Statistical Analysis
Clinical signs of disease were most prominent on days 3 through 6 after challenge with BHV1 in the nonvaccinated group. Therefore, to determine if there were significant differences between treatment groups, the composite clinical scores for days 3 through 6 were compared using an analysis of variance procedure with day after infection as a blocking factor using SAS software (SAS, Cary, NC). Least significant difference at $P < .05$ was then used to determine statistically significant differences between groups.

RESULTS
The results for body temperature and composite clinical score for the groups vaccinated 14 days before challenge are found in Figures 1A and 1B. Results for the groups vaccinated 5 days before challenge are found in Figures 2A and 2B. The composite clinical score for days 3 through 6 provides the best summary of overall level of protection by the vaccines and is therefore presented graphically in Figures 3A and 3B. All four vaccine combinations used provided solid protection from BHV1 challenge when administered 14 days before challenge. There was no significant difference between them ($P > .05$). All four vaccines used provided significant ($P < .05$) protection compared with the nonvaccinated group when given 5 days before challenge; however, some provided better protection than others. Vaccine A provided significantly ($P < .05$) better protection than vaccine B or vaccines B plus D when given 5 days before challenge. Vaccine B and vaccines B plus C provided significantly ($P < .05$) better protection than vaccines B plus D.

Vaccine A and vaccines B plus C provided essentially equivalent protection at 5 days and 14 days after vaccination (Figures 3A and 3B). Vaccine B and vaccines B plus D provided significantly ($P < .05$) better protection at 14 days after vaccination than at 5 days after vaccination.

The results of BHV1 isolation from nasal secretions are found in Figures 1C and 2C. The nonvaccinated calves (group 9) had detectable virus in the nasal secretions from days 2 through 10 after challenge with peak shedding on day 4. All of the vaccinated groups of calves had less viral shedding than the nonvaccinated group (group 9). All of the vaccines, when administered 14 days before challenge, nearly eliminated virus shedding in the nasal secretions. The vaccines were somewhat less effective at preventing viral shedding when given 5 days before challenge, especially vaccines B plus D.

DISCUSSION
All of the vaccination regimens used induced solid protective immunity at 14 days after vaccination with no significant difference between them. All of the vaccination regimens used also induced statistically significant protective immunity at 5 days after vaccination; however, there were significant differences in the degree of protection afforded by the various vaccination protocols. The best protection induced by 5 days after vaccination was provided by the MLV + KV combination vaccine (vaccine A) injected IM and the MLV vaccines given IN + IM concurrently (vaccines B + D). The MLV + KV combination vaccine (vaccine A) administered IM gave significantly better protection than the MLV vaccine administered IN (vac-
Figure 1A

Figure 1B

Figure 1C

Figure 1. Mean rectal temperature (1A), composite clinical score (1B), and isolation of BHV1 in nasal secretions (1C), from calves challenged 14 days after vaccination.
Figure 2A

Figure 2B

Figure 2C

Figure 2. Mean rectal temperature (2A), composite clinical score (2B), and isolation of BHV1 in nasal secretions (2C), from calves challenged 5 days after vaccination.
cine B). Administering a KV BHV1 vaccine (vaccine D) IM at the same time as the MLV BHV1 vaccine (vaccine B) IN reduced the effectiveness of the IN vaccine for inducing early protection (Figures 3A and 3B). Early onset of protection after vaccination against BHV1 can be caused by a combination of induction of cytokines (including interferon), induction of systemic or intranasal antibody, activation of T lymphocyte subsets, activation of natural killer lymphocytes, and perhaps other mechanisms. The mechanisms responsible for the more rapid induction of immunity with the various vaccination regimens used is unknown.

The best protection at 5 days after vaccination was afforded by the two vaccines that included MLV vaccine administered IM. One of the two vaccines also included killed BHV1 in the vaccine formulation IM; the other vaccination regimen included a concurrent administration of MLV IN vaccine. Because an MLV vaccine administered IM by itself was not test-
ed in this experiment, the authors cannot be certain that the other two components contributed to more rapid onset of protective immunity. It is possible that an MLV IM vaccine administered by itself would provide equivalent protection at 5 days after vaccination.

It is interesting to note that one of the vaccines that induced the best protection at 5 days after vaccination contained both MLV and KV BHV1 administered IM, whereas a KV BHV1 vaccine significantly \( (P < .05) \) reduced the efficacy of a concurrently administered MLV IN vaccine. This difference could be because of the difference in route of administration of the MLV vaccines (IM versus IN) or to differences in adjuvants or other properties of the different KV vaccines used.

The calves used in this experiment were seronegative for BHV1 at the time of vaccination. Therefore, these results are valid for calves that do not have maternal antibody against BHV1. Calves with maternal antibody can respond differently to the vaccines; however, calves with maternal antibody already have some protection against BHV1 and do not require rapid onset of active immunity in order to be protected during the first few days after vaccination.

**CONCLUSION**

All vaccine regimens in this study induced solid protection against BHV1 challenge at 14 days after vaccination. All of the vaccine regimens also induced statistically significant \( (P < .05) \) protective immunity at 5 days after vaccination; however, there were significant differences \( (P < .05) \) in the degree of protection. The best protection induced by 5 days after vaccination was provided by the MLV + KV combination vaccine injected IM and the MLV vaccines given IN and IM concurrently. The MLV + KV combination vaccine administered IM gave significantly \( (P < .05) \) better protection by 5 days after vaccination than the MLV vaccine administered IN. Administering a KV BHV1 vaccine IM at the same time as the MLV BHV1 IN vaccine significantly \( (P < .05) \) reduced the effectiveness of the IN vaccine for inducing early protective immunity.

**REFERENCES**

11. Savan M, Angulo AB, Derbyshire JB: Interferon, antibody responses and protection induced by an in-

