Evaluation of the Efficacy of a Modified-Live Combination Vaccine against Abortion Caused by Virulent Bovine Herpesvirus Type 1 in a One-Year Duration-of-Immunity Study*

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This study demonstrated that a multivalent vaccine containing modified-live bovine herpesvirus type 1 (BHV-1) protected pregnant heifers and their fetuses against virulent BHV-1 challenge exposure at 365 days after vaccination. The percentage of abortions or fetal deaths caused by BHV-1 was significantly higher in control heifers (10 of 10 [100.0%]) than BHV-1–vaccinated heifers (three of 19 [15.8%]).

INTRODUCTION

Of the reproductive problems in male and female cattle caused by bovine herpesvirus type 1 (BHV-1)—temporary infertility, abortion, infectious pustular vulvovaginitis, and infectious balanoposthitis—the greatest economic threat comes from losses resulting from BHV-1–induced abortions, which occur chiefly during the last half of gestation, often without evidence of other clinical signs.1,2 The estimated 25% of susceptible cows aborting because of BHV-1 represents significant loss in terms of both valuable genetic potential as well as market value.3 In affected beef herds, abortions usually occur during a 3- to 4-month period, during which time 50% or more of the cows may abort.4 In dairy herds, BHV-1–induced abortion storms can last a year or more, compromising profits through calf death, loss of genetic potential, and failure to reach peak lactation.4

BHV-1 infection can occur at conception as a result of infected semen or infected embryos at transplant. In such cases, the virus infects the embryo at about the eighth or ninth day of gestation. Additionally, the virus can cause lesions in the uterus, ovaries, and corpus lu-
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BHV-1–induced abortions represent a serious economic threat in both beef and dairy herds.

quentlly, the dead fetus is retained in utero for 2 or more days and usually exhibits some degree of autolysis at expulsion. The placenta is often retained after the birth or abortion of an infected fetus.

To help protect against BHV-1 abortion, it is essential that BHV-1 vaccines provide fetal protection. Only a few inactivated virus and modified-live virus (MLV) vaccines have been shown to induce fetal protection against experimental challenge. In one study, nine of 10 heifers vaccinated with a temperature-sensitive MLV vaccine were protected against an intravenous challenge with BHV-1 that caused abortions or stillbirths in 10 of 10 nonvaccinated control heifers. While existing BHV-1 vaccines have label claims for providing protection against the respiratory forms of BHV-1, most do not have claims for protection against the reproductive forms. Thus, properly administered vaccination programs can be counted on to protect the cow against severe disease and death, but they may not always protect the fetus. Currently, BHV-1 vaccines licensed in the United States are not required to provide fetal protection. The MLV BHV-1 vaccine component evaluated in this report has a label claim for aiding in the prevention of abortion induced by BHV-1.

The purpose of the study reported here was to evaluate the efficacy of a freeze-dried MLV BHV-1–bovine viral diarrhea virus (BVDV) types 1 and 2–parainfluenza virus-3 (PIV3)–bovine respiratory syncytial virus (BRSV) vaccine reconstituted with an inactivated bacterin containing Campylobacter fetus; Leptospira interrogans serovars Canicola, Icterohaemorrhagiae, Hardjo (hardjoprajitno), and Pomona; and Leptospira kirschneri serovar Grippotyphosa (Bovi-Shield GOLD FP 5 VL5, Pfizer Animal Health) against a virulent BHV-1 challenge administered 365 days after a single-dose vaccination.

MATERIALS AND METHODS

Study Animals

Procedures involving cattle were approved by the Pfizer Committee for Animal Care and Use. Sixty-two commercial beef heifers (9 to 12 months of age) with no history of vaccination with any product containing BHV-1 were obtained from a single source and enrolled in the study (Table 1). The heifers were confirmed to be seronegative to BHV-1 (serum virus neutralizing [VN] antibody titers <2) and negative for persistent BVDV infection by earnotch immunohistochemistry testing before being enrolled in the study. Each animal was identified with a uniquely numbered ear tag.
and was randomly assigned to the placebo control ($n = 22$) or BHV-1–vaccinated ($n = 40$) group before vaccination on study day 0. The two groups were maintained on separate isolated pastures until challenge. On day 28, two animals from the placebo control group were randomly selected and moved to the pasture with the BHV-1–vaccinated group to serve as serologic sentinels. The two sentinels remained with the BHV-1–vaccinated group until relocation to the challenge site on day 356. All animals had free access to feed and water throughout the study.

After estrus synchronization, all study heifers were bred by artificial insemination on day 150 with semen certified to be free of BHV-1 and BVDV. The animals were subsequently observed for heat and, if detected, were rebred through day 172. Rectal palpation on day 336 confirmed pregnancy in 13 placebo control heifers and 21 vaccinates. On day 356, 10 placebo controls and 20 BHV-1–vaccinated animals were randomly selected for challenge and transported separately to the challenge site, where the animals were held throughout the remainder of the study. Control and vaccinated groups were commingled on the day of challenge (day 365) and were kept together through calving. Heifers were allowed to calve naturally.

### Vaccine
A multivalent vaccine consisting of modified-live BHV-1, BVDV (types 1 and 2), PIV, and BRSV was used. The freeze-dried viruses were rehydrated with an inactivated bacterin containing C. fetus; L. interrogans serovars Canicola, Icterohaemorrhagiae, Hardjo (hardjoprajitno), and Pomona; and L. kirschneri serovar Grippotyphosa (Bovi-Shield GOLD FP 5 VL5). The placebo control (Vibrio/Leptoferm 5 [Pfizer Animal Health]) consisted of the bacterin alone. Both vaccines were formulated using antigens at release levels, except for the modified-live BHV-1 fraction of the test vaccine, which was added at the minimum immunizing dose (MID) level. An MID defines the maximum virus passage and minimum dosage content required to stimulate immunity. Release levels of antigen are substantially higher than MID and ensure that adequate antigen content is maintained throughout the product’s shelf-life. The vaccines were administered intramuscularly in 2-ml doses.

### Challenge
On day 365, the heifers were inoculated by the intravenous route with a 2-ml dose containing $6.8 \log_{10} \text{TCID}_{50}$ (median tissue culture infective dose) of virulent BHV-1 Cooper strain. The heifers were between 193 and 215 days of gestation on the day of challenge. The virus titer

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### TABLE 1. Experimental Design of a 1-Year Duration-of-Immunity Study Evaluating Fetal Protection against Virulent BHV-1 Challenge

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Treatment (Route)</th>
<th>No. of Doses/Challenge Route</th>
<th>No. of Animals Selected for Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo control ($n = 22$)</td>
<td>C. fetus–5-way Leptospira (IM)</td>
<td>1 IV</td>
<td>10</td>
</tr>
<tr>
<td>BHV-1 vaccinated ($n = 40$)</td>
<td>BHV-1–BVDV–PIV$_3$–BRSV–C. fetus–5-way Leptospira (IM)</td>
<td>1 IV</td>
<td>20</td>
</tr>
</tbody>
</table>

*Heifers were challenged IV on day 365 with $6.8 \log_{10} \text{TCID}_{50}$ (median tissue culture infective dose)/2-ml dose of the Cooper strain of BHV-1.
was determined for the challenge as described previously, except that five replicate dilutions (eight wells/dilution) were made and plates were incubated for 7 days before evaluation of cytopathic effect for determining end-point titer.

**Heifer Assessments**

Heifers were monitored for general health at least once weekly before challenge and daily after challenge throughout the study. All assessments conducted during the challenge phase of the study were performed without knowledge of treatment group assignment. Rectal temperatures were measured and recorded on day 365 (before challenge), daily from day 369 through day 373, and on day 379. Blood samples were collected from each heifer on study days 0, 28, 56, 112, 175, 224, 280, 336, 365 (before challenge), and 386 for determination of serum VN antibody titers to BHV-1. Virus isolation (VI) of BHV-1 was conducted on serum harvested from blood samples obtained from each heifer on days 0, 365 (before challenge), 369 through 373, and 379.

Blood samples were submitted to Benchmark BioLabs (Lincoln, NE) for processing and subsequent testing. The VN assay utilized 96-well plates containing Madin-Darby bovine kidney (MDBK) cells. The cell growth medium was Dulbecco’s modified Eagle’s medium supplemented with L-glutamine, gentamicin, nonessential amino acids, fetal bovine serum, and amphotericin B. Each serum sample was diluted twofold in growth medium, an appropriate dilution of the Cooper strain of BHV-1 was added, and the mixture was incubated at room temperature for 45 minutes before being transferred to a 96-well plate containing MDBK cells. The plates were incubated at 37°C with 5% carbon dioxide for 6 (±1) days and read for cytopathic effects typical of BHV-1. Each serum sample to be tested for VI was added to eight wells of a 96-well plate containing MDBK cells in the same medium used for VN testing. The plates were incubated and read for cytopathic effects typical of BHV-1, as indicated above.

**Fetal Assessments**

After the challenge, heifers were observed daily for abortions. All aborted fetuses and dead calves were submitted to the Illinois Department of Agriculture, Bureau of Animal Disease Laboratory (Galesburg, Ill), for necropsy and diagnostic evaluation. VI, fluorescent antibody testing, and histologic examinations were performed on fetal tissues from aborted or stillborn animals following the standard procedures used at the diagnostic laboratory. All observations and laboratory evaluations were conducted without knowledge of treatment group assignments.

**Statistical Analysis**

Rectal temperatures were analyzed with a general linear repeated measures mixed model. Contrasts of interest were made after detecting a significant \( P \leq .05 \) treatment or treatment by time period interaction. Fisher’s exact test was used to compare the rate of fetuses affected by challenge and dams positive for VI (at least 1 day after challenge) between treatment groups. A determination of the cause of each aborted, weak, or stillborn calf was made using routine diagnostic techniques to determine whether the fetus was affected by challenge.

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**To help protect against BHV-1 abortion, it is essential that BHV-1 vaccines provide fetal protection.**

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The percentage of days following challenge that each dam was positive for VI was compared between treatment groups, with the analysis conducted using a general linear mixed model. For each animal, the percentage of days postchallenge positive was converted to a proportion and transformed using the arcsine of square root before analysis. After analysis, the least squares means were back-transformed to percents. Serum VN titers were log transformed before calculation of descriptive means for each treatment by day of study. Means were back-transformed and presented as geometric means.

### RESULTS

#### Heifer Assessments

The least squares mean rectal temperatures of placebo control group heifers were significantly ($P \leq .0412$) higher than the temperatures of the BHV-1–vaccinated heifers on days 369, 370, 371, and 373 (Table 2).

All 30 challenged animals were seronegative (VN titer <2) for antibodies to BHV-1 on study day 0, and all 10 placebo control animals remained seronegative up to the day of challenge (Table 3). The two sentinel heifers also remained seronegative, except on day 56 when one of the heifers had a titer of 2. Titers for this animal at the subsequent five time points were below 2 (data not shown). After vaccination, the BHV-1–vaccinated animals developed antibody titers that persisted through challenge on day 365. On day 386 (21 days after challenge), the BHV-1 serum VN geometric mean antibody titers were increased in both study groups, indicating successful challenge of the control heifers and an anamnestic response (2.4-fold increase) in the BHV-1 vaccinates.

The placebo control group had a significantly ($P \leq .00985$) higher number (three of 10) of animals with BHV-1 isolated from sera following challenge than the BHV-1–vaccinated group (Table 4). Viremia was not detected in any of the 20 BHV-1–vaccinated heifers at any of the six sampling days after challenge.

#### Fetal Assessments

Within 66 days of BHV-1 challenge, 13 heifers aborted spontaneously and one heifer was induced to abort a nonviable fetus (Table 5). One fetus from a BHV-1–vaccinated heifer was excluded from analysis; this heifer aborted within 24 hours of challenge, and fetal examination failed to identify the presence of BHV-1 but did reveal a ruptured fetal liver. The rupture probably resulted from incidental physical trauma the dam experienced during handling at the time of challenge and likely was the cause of the abortion.

One hundred percent (10 of 10) of the fetuses from placebo control heifers were confirmed positive for BHV-1 by histologic exam-
ination and/or VI. One aborted fetus from the vaccinated heifers showed histologic evidence of BHV-1 infection. Fetuses recovered from two additional BHV-1–vaccinated heifers were badly decomposed at the time of discovery, and samples were not available for submission to the diagnostic laboratory. Although a definitive diagnosis could not be made on these two fetuses, the abortions were considered to be a result of the BHV-1 challenge; therefore, 84.2% (16 of 19) of the fetuses from heifers in the vaccinated group were protected. The difference between the placebo control and BHV-1–vaccinated groups in the percentage of animals aborting due to BHV-1 infection was significant ($P \leq .0001$).

### DISCUSSION

Little is known about the duration of the protective immunity of BHV-1 vaccines. An intranasally administered modified-live BHV-1 vaccine provided protection to pregnant animals that were challenged 316 days after vaccination. Recently, a combination BHV-1 vaccine was demonstrated to protect calves from infection with virulent virus for at least 126 days following vaccination. The combination vaccine evaluated in the current study contains a modified-live strain of BHV-1 and is registered with the regulatory authorities for vaccination of healthy cows and heifers before breeding as an aid in preventing abortion caused by BHV-1. Results provide evidence

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**TABLE 3. Geometric Mean BHV-1 Serum Virus-Neutralizing Antibody Titers**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day of Study</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 28 56 112 175 224 280 336 365* 386</td>
</tr>
<tr>
<td>Placebo control (n = 10)</td>
<td>1.0* 1.0 1.0 1.0 1.0 1.0 1.0 1.0 113.5</td>
</tr>
<tr>
<td>BHV-1 vaccinated (n = 20)</td>
<td>1.0 23.0 17.7 14.9 15.0 13.7 20.5 28.4 49.6 121.5</td>
</tr>
</tbody>
</table>

*Pregnant heifers were challenged on day 365.
*Titers <2 were analyzed as 1.0.

**TABLE 4. Percentage of Heifers Positive for Viremia by Day and Total Percentage Positive across All Days Following BHV-1 Challenge**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Percent Serum BHV-1 Positive by Day of Study</th>
<th>Percent of Heifers with at Least One Positive Virus Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>365a 369 370 371 372 373 379</td>
<td></td>
</tr>
<tr>
<td>Placebo control (n = 10)</td>
<td>0 20 20 20 10 0 0</td>
<td>30b</td>
</tr>
<tr>
<td>BHV-1 vaccinated (n = 20)</td>
<td>0 0 0 0 0 0 0</td>
<td>0c</td>
</tr>
</tbody>
</table>

aPregnant heifers were challenged on day 365.
b,c Values within the same column with different lower-case superscripts are significantly ($P \leq .00985$) different.
that vaccination establishes long-term immunity, as pregnant heifers and their fetuses were protected against virulent BHV-1 challenge administered 365 days after vaccination. The study was conducted for the purpose of obtaining a 1-year duration of immunity label indication for the combination vaccine’s BHV-1 component. Accordingly, the BHV-1 fraction contained only the MID level intended to put the challenged vaccine component at maximum potential disadvantage.

In the current study, all 10 placebo control heifers remained seronegative for antibodies to BHV-1 from the day of vaccination until after challenge. In contrast, all BHV-1–vaccinated heifers seroconverted to BHV-1 after vaccination. The geometric mean antibody titers of BHV-1–vaccinated heifers peaked and remained relatively unchanged before challenge; however, an anamnestic response was detected following challenge, indicating immunologic memory in the vaccinates. The two groups of heifers were kept separate during the prechallenge phase of the study to minimize any potential exposure to the susceptible controls if the vaccine virus was shed. The lack of seroconversion in the sentinel heifers indicated that the vaccine virus was not shed and that there was no additional exposure to BHV-1 until challenge on day 365.

Efficacy of the BHV-1 vaccination was confirmed on the basis of significant differences between placebo control and BHV-1–vaccinated groups in rectal temperatures, serum VI results, and number of aborted calves attributable to BHV-1 infection. After challenge, placebo control heifers had elevated temperatures of several days’ duration, and their temperatures were significantly ($P \leq .0412$) higher than those of BHV-1–vaccinated heifers. The peak rectal temperatures following challenge in the control heifers were similar to those observed previously using this challenge method. Additionally, BHV-1 was isolated from a significantly ($P \leq .00985$) higher percentage (30%) of placebo control heifers than BHV-1–vaccinated heifers (0%). It is likely that more animals were viremic following challenge, but since viremia was not the primary variable of the study, only a few postchallenge days were monitored to minimize the stress of additional handling and sampling on the pregnant heifers. Peak shedding typically occurs 4 to 8 days after intranasal challenge with BHV-1. In contrast, this period might not be the best time to detect viremia when virus is administered intravenously. More study is needed to characterize viremia following intravenous challenge.

The most dramatic and important difference between groups, however, was evident in the significant ($P \leq .0001$) degree of fetal protection established by vaccination. Whereas 0 of 10 fetuses (0%) from placebo control heifers were protected against BHV-1–induced abortion, 84.2% (16 of 19) of the fetuses from BHV-1–vaccinated heifers were protected. The challenge model used in this study has previously been proven to be effective in inducing abortions in susceptible animals. In that study, the controls aborted or delivered stillborn calves within 20 to 79 days after challenge. Nine of 10 controls in the current study aborted 17 to 29 days after challenge, and the tenth animal had to be induced to abort 66 days after challenge following detection of a nonviable fetus.

<table>
<thead>
<tr>
<th>Table 5. Number and Percentage of Abortions Attributed to BHV-1 Challenge</th>
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<tbody>
<tr>
<td>Treatment Group</td>
</tr>
<tr>
<td>Placebo control ($n = 10$)</td>
</tr>
<tr>
<td>BHV-1 vaccinated ($n = 20$)</td>
</tr>
</tbody>
</table>

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In the current study, a single dose of vaccine given 1 year before challenge protected pregnant heifers against abortion. The 365-day duration of immunity documented in this study complements previous demonstration of the BHV-1 vaccine’s safety in pregnant animals that had been previously vaccinated in accordance with label directions. Incorporation of the vaccine into a comprehensive health program may significantly advance efforts directed toward controlling BHV-1 herd infections and associated economic losses.

CONCLUSION

The modified-live BHV-1 fraction of a combination vaccine significantly \((P \leq .0001)\) protected pregnant heifers against abortion following intravenous inoculation with virulent BHV-1 365 days after vaccination.

REFERENCES