

# Efficacy of bovine herpesvirus-1 inactivated vaccine against abortion and stillbirth in pregnant heifers

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**Objective**—To evaluate the efficacy of an inactivated bovine herpesvirus-1 (BHV-1) vaccine to protect against BHV-1 challenge-induced abortion and stillbirth.

**Design**—Prospective study.

**Animals**—35 beef heifers.

**Procedures**—Before breeding, heifers were vaccinated with a commercially available BHV-1 inactivated vaccine SC or IM. The estrous cycle was then synchronized, and heifers were artificially inseminated 30 to 60 days after vaccination. Heifers (n = 21) were challenge inoculated IV at approximately 180 days of gestation with virulent BHV-1. Fourteen control heifers were not vaccinated. Clinical signs of BHV-1 infection were monitored for 10 days following challenge; serologic status and occurrence of abortion or stillbirth were evaluated until time of calving.

**Results**—18 of 21 (85.7%) heifers that received vaccine were protected from abortion following challenge, whereas all 14 control heifers aborted.

**Conclusions and Clinical Relevance**—Results indicated that an inactivated BHV-1 vaccine can protect against abortion resulting from a substantial challenge infection, with efficacy similar to that of modified-live BHV-1 vaccines. (*J Am Vet Med Assoc* 2007;231:1386–1389)

Infectious bovine rhinotracheitis is a clinically and economically important disease of cattle and is endemic in cattle populations throughout the world. Infectious bovine rhinotracheitis caused by BHV-1 is associated with a variety of clinical signs and can cause respiratory as well as reproductive disease. Bovine herpesvirus type 1 is often associated with the bovine respiratory disease complex and can also predispose animals to secondary bacterial infections. Bovine herpesvirus type 1 is spread through nasal secretions, droplets, genital secretions, serum, and fetal fluids.<sup>1</sup>

Nonvaccinated pregnant cattle are susceptible to the reproductive effects of BHV-1, and infections can result in abortion rates as high as 25%. These infections can also result in late-term abortions that can occur up to 100 days after infection.<sup>2</sup>

Vaccination with either an MLV or inactivated vaccine is the most effective way to control the spread of BHV-1. Modified-live BHV-1 vaccines are administered parenterally (SC or IM) or IN, whereas inactivated vaccines are administered SC or IM. However, there have been adverse effects associated with modified-live BHV-

## ABBREVIATIONS

BHV-1	Bovine herpesvirus type 1
MLV	Modified-live virus
IN	Intranasal
BVDV	Bovine viral diarrhea virus

1 vaccines, including abortion in pregnant animals with unknown or questionable vaccine status.<sup>3–5</sup>

Five BHV-1 reproduction protection studies have been reported in the literature. Four of those studies have tested MLV vaccine efficacy.<sup>6–9</sup> Only 1 study<sup>10,11</sup> with an inactivated vaccine has been reported.

The objective of the study reported here was to evaluate the efficacy of an adjuvanted, inactivated BHV-1 vaccine administered prior to breeding as a prophylactic treatment against abortion caused by IV challenge with virulent BHV-1 in cattle.

## Materials and Methods

**Animals**—Protocols were reviewed and approved by the Rural Technologies Incorporated Institutional Animal Care and Use Committee. Thirty-five 6- to 9-month-old beef (Angus cross) heifers were acquired for the study and randomly assigned to 1 of 3 treatment groups. Heifers in treatment group 1 (n = 12) were designated as IM vaccinates, heifers in treatment group 2 (9) were designated as SC vaccinates, and heifers in treatment group 3 (14) were sham vaccinated and designated as control heifers. Heifers were managed according to routine animal husbandry procedures and were isolated from any other cattle.

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**Prevaccination serologic assays**—Blood was collected from all heifers prior to vaccination. All heifers were seronegative for antibodies against BHV-1 and BVDV and were negative to BVDV via ear notch testing. Serum samples were tested for BHV-1 and BVDV serum neutralizing antibody titers by use of the constant virus decreasing serum assay. Two-fold serial dilutions (range, 1:2 to 1:1,024) of sera in quadruplicate were incubated with a constant viral titer ( $< 500$  TCID<sub>50</sub>) before inoculation of Madin-Darby bovine kidney cells in microtiter tissue culture plates. Plates were incubated at 37°C with 5% CO<sub>2</sub> for 4 to 6 days for BHV-1 and 5 to 7 days for BVDV before being evaluated for virus-induced cytopathic effect. The reciprocal of the last dilution that prevented cytopathic effect was designated the serum neutralizing antibody titer. Geometric mean values were calculated by use of log<sub>2</sub> titers.

**Vaccination**—Thirty-five heifers were vaccinated at approximately 1 year of age (day 0). Twelve heifers in group 1 were vaccinated IM and 9 heifers in group 2 were vaccinated SC by use of a commercially available inactivated combination vaccine<sup>a</sup> according to manufacturer's recommendations. The remaining 14 control heifers in group 3 were sham vaccinated with an oil-adjuvanted vaccine that did not contain viral antigens. All heifers were booster vaccinated with the appropriate vaccine on day 29. Heifers were observed daily after each vaccination for vaccine-related adverse events.

**Synchronization and breeding**—The heifers' estrous cycles were synchronized by use of a vaginal implant,<sup>b</sup> a gonadotrophin-releasing hormone, and prostaglandin. Implants were inserted vaginally, and gonadorelin diacetate tetrahydrate<sup>c</sup> was administered IM. The implants were removed, detectors<sup>d</sup> were placed on the tail-head area to aid in estrus detection, and the heifers were administered dinoprost tromethamine<sup>e</sup> IM. Heifers were observed twice daily for signs of estrus (color change in detector from white to red), and heifers with a red detector were artificially inseminated 12 hours later. At breeding, the estrus detector was removed. Two virgin BHV-1 vaccinated clean-up bulls were put in with the heifers for 3 weeks following artificial insemination. The bulls had been vaccinated with a commercially available inactivated viral vaccine<sup>f</sup> at 2, 5, and 7 months of age and were revaccinated<sup>a</sup> at 11 months of age with the same product used on the heifers. The bulls were purchased at 15 months of age and held in separate facilities for approximately 2 months prior to exposure to the heifers. Semen from both clean-up bulls yielded negative results for BVDV and BHV-1 by use of a PCR assay and negative results for BVDV by use of ear notch testing with an ELISA. A semen sample from the bull used for artificial insemination also yielded negative results for BVDV by use of a PCR assay. Heifers were palpated transrectally prior to challenge to confirm pregnancy status.

**Challenge inoculation**—All heifers received an IV challenge with 2 mL of BHV-1 (Cooper strain,<sup>g</sup> approx  $3 \times 10^6$  TCID<sub>50</sub>/mL) at approximately 180 days of gestation and 230 days after the second vaccination. Clinical observations were performed daily from 2 days prior to

challenge through day 10 after challenge. Each heifer was visually examined in the pen prior to handling and scored for clinical signs, including abnormal respiration, nasal and ocular discharge, nasal lesions, cough, and attitude, by use of a scale of 0 to 3, with the absence of a clinical sign scored as 0 and the most severe clinical sign scored as 3. After visual assessment, heifers were restrained and rectal temperatures<sup>h</sup> were determined. All heifers were observed daily for signs of abortion from the time of challenge through the time of calving.

**Serologic testing**—Blood was collected via jugular venipuncture from the heifers prior to each vaccination, prior to challenge, 10 days following challenge, and 64 days following challenge. Serum neutralizing antibody titers against BHV-1 were determined by use of the constant virus decreasing serum assay. Blood was also collected via jugular venipuncture from neonatal calves of the vaccinated heifers prior to colostrum ingestion and tested for BHV-1 neutralizing antibody.

**Fetal tissue collection and testing**—Samples were collected from aborted fetuses and tested for BHV-1 and BVDV. Prior to testing, fetal spleen samples were matched to the appropriate dam via tail switch hair samples by use of DNA parentage testing.<sup>i</sup> Heart blood, pleural fluid, or both were tested for neutralizing antibody titers against BHV-1 and BVDV. Lung, placenta, and stomach contents were tested for abortagenic bacteria, and a kidney sample was tested for leptospiral organisms via fluorescent antibody testing.<sup>j</sup> Thymus, lung, liver, spleen, kidney, and brain were each tested for BHV-1 and BVDV via virus isolation.<sup>k</sup> Briefly, dilutions of processed samples were made, and each diluted sample was added in triplicate for BHV-1 and in quadruplicate for BVDV to BVDV-free bovine turbinate cell monolayers in microtiter tissue culture plates. The BHV-1 culture plates were incubated for 2 to 3 days at 37°C with 5% CO<sub>2</sub>. For BVDV, the culture plates were incubated for 3 to 4 days at 37°C with 5% CO<sub>2</sub>, followed by 2 additional passages incubated for 3 to 4 days each. Results were considered positive if BHV-1 or BVDV virus-specific staining was observed in inoculated cells.

**Statistical analysis**—The Fisher exact test was used to test the hypothesis of no difference in frequency of abortion or stillbirths between the 2 vaccinated groups (SC vs IM); the results indicated no significant differences, and therefore, the 2 vaccine groups were combined for all further analyses.

The proportion of animals that aborted was analyzed by use of the Fisher exact test. Temperatures were compared between the control and vaccinated groups by use of ANCOVA, and serum neutralization data were analyzed by use of ANCOVA and ANOVA. Clinical scores between the control and the vaccinated groups were evaluated by use of a parametric repeated-measures ANCOVA. For all comparisons,  $P < 0.05$  was considered significant.

## Results

**Clinical observations**—Adverse vaccine reactions were not observed in any heifers. Following challenge, body temperature was measured rectally in all heifers from

2 days prior to challenge to 10 days after challenge, with the mean determined for each group (Figure 1). Control heifers had higher mean temperatures than vaccinates over the entire course of the trial. Significant ( $P < 0.001$ ) differences were detected on days 2 to 5 after challenge, with the mean temperature in the control group higher than 39.7°C (103.5°F [study cutoff value for pyrexia]) on days 2 (39.9°C [103.8°F]), 3 (39.8°C [103.6°F]), and 4 (40.4°C [104.7°F]).

Clinical observation scores were totaled for each heifer beginning 2 days prior to challenge through 10 days after challenge, and the mean of these composite scores was determined for each group (Figure 2). Control heifers (group 3) had significantly ( $P = 0.03$ ) higher mean clinical scores than vaccinates from 7 to 10 days after challenge. Mean clinical scores for group 1 peaked on days 4 and 6 after challenge; those in group 2 peaked on days 4, 6, and 7 after challenge.

**Fetal loss**—Seventeen heifers aborted following challenge. Three of 21 (14.3%; 1 from the SC group and 2 from the IM group) heifers from the vaccinated group and 14 of 14 heifers in the control group aborted. The vaccinated group had significantly ( $P \leq 0.001$ ) fewer abortions, compared with the control group.

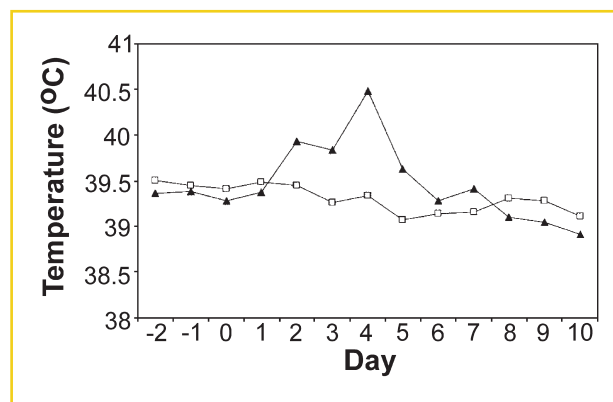


Figure 1—Mean rectal temperatures after challenge with BHV-1 in control heifers (triangles) and heifers vaccinated with an inactivated vaccine (squares), measured 2 days prior to challenge (day -2) until 10 days after challenge. Difference between groups was significant ( $P < 0.05$ ) on days 2, 3, 4, 5, and 7.

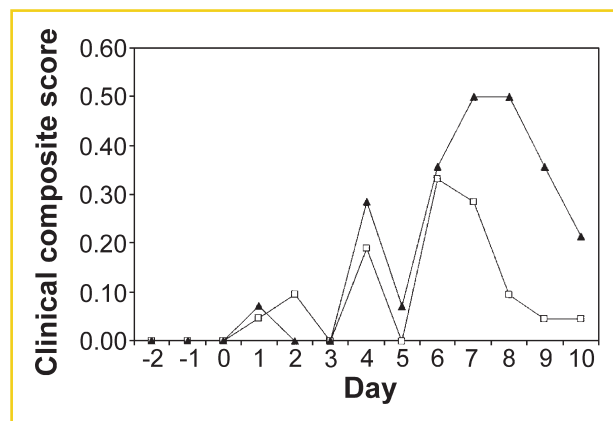


Figure 2—Mean clinical composite scores after challenge with BHV-1 in control heifers (triangles) and heifers vaccinated with an inactivated vaccine (squares). Difference between groups was significant ( $P < 0.05$ ) on days 7 through 10.

**Serum neutralizing antibody titers**—All heifers had titers of 0 at the time of the first vaccination. Heifers in the vaccinated group had significantly ( $P < 0.001$ ) higher mean titers (2.71) than did control heifers (0.09; 1 heifer had a titer of 2.3) on the day of second vaccination. On the day of challenge, vaccinated heifers had a mean titer of 5.10, which was significantly ( $P < 0.001$ ) higher than that of control heifers, of which 2 had a mean titer of 0.16 (1 had a titer of 1.3 [the animal with a titer of 2.3 at the second vaccination], and 1 had a titer of 1.0). On day 10 following challenge, vaccinated heifers had a mean titer of 6.58, and the control heifers had a mean titer of 3.81. On day 64 following challenge, vaccinated heifers had a mean titer of 6.47 and the control heifers had a mean titer of 6.38.

**Fetal tissue results**—Tissues from all aborted fetuses yielded positive results for BHV-1 via virus isolation and fluorescent antibody testing. The fetal tissues yielded negative results for BVDV via virus isolation, negative results for *Leptospira* spp via fluorescent antibody assay, and negative results for bacterial pathogens via culture.

**Neonatal calves**—All calves that had not suckled ( $n = 16$ ) yielded negative results for anti-BHV-1 antibodies. Two of the neonatal calves suckled prior to sample collection and were excluded from the serologic analysis.

## Discussion

Results indicated that an inactivated multivalent vaccine<sup>a</sup> containing BHV-1 administered before breeding protected against abortion despite a virulent BHV-1 challenge at approximately 180 days of gestation. Vaccinated heifers had fewer clinical signs, lower rectal temperatures, and significantly fewer abortions. Among vaccinated heifers, 85.7% were protected against abortion, whereas all control heifers (14/14) aborted following challenge. Bovine herpesvirus type 1 was isolated from all aborted fetuses, and no other pathogens were detected. Additionally, positive identification of heifer-fetus pairs was accomplished via DNA parentage testing.

The ability of an inactivated vaccine to protect heifers from abortion is important because inactivated vaccines can be administered during gestation and lactation. Some MLV vaccines are presently approved for administration during gestation and lactation; however, abortions can occur when these vaccines are administered to animals with unknown or questionable vaccine status.<sup>3-5</sup>

Several BHV-1 reproduction protection studies<sup>6-9</sup> that used MLV vaccines and a single study<sup>10,11</sup> on inactivated vaccines can be found in the literature. However, challenge models and study designs are not consistent among studies, making it difficult to compare results from those studies with results of the present study. Three studies used MLV vaccine administered IM prior to breeding, followed by either an IN challenge<sup>6</sup> or an IV challenge.<sup>8,9</sup> One study<sup>7</sup> used an IN administered vaccine prior to breeding, followed by an IN challenge. Only 1 study<sup>10,11</sup> that used an inactivated vaccine has been reported in the literature to our knowledge. That study involved vaccinating bred cattle from seropositive or seronegative herds and used either an intratracheal or IV challenge.



In the study reported by Saunders et al,<sup>6</sup> heifers were vaccinated IM once or twice with an MLV and challenged IN at either 3, 4, 5, or 6 months of gestation. After challenge, 10 of 16 control heifers and 1 of 17 vaccinates aborted. However, BHV-1 was isolated from only 3 placentas and 1 fetal tissue sample. In the study by Smith et al,<sup>7</sup> heifers were vaccinated once IN with an MLV vaccine and challenged IN at 7.5 to 9 months of gestation. Twelve of 16 control heifers had fetal loss following challenge, and BHV-1 was isolated from 11 of the 12 fetuses. In contrast, in the study reported here, all control heifers aborted. If the severity of challenge in the previous studies had been higher, it is possible that the level of protection in vaccinates would have been comparable to our study.

Additional studies of MLV vaccines have used a challenge model and a study design comparable to those in the present study. In a study by Cravens et al,<sup>8</sup> an MLV vaccine was administered IM 2 times before breeding and heifers were challenged at approximately 6 months of gestation. Nine of 10 vaccinated heifers were protected from BHV-1-induced abortion, whereas all 10 control heifers had fetal loss (8 abortions and 2 stillbirths). Interestingly, no BHV-1 was isolated from any of the aborted or stillborn fetuses, although histologic findings and results of BHV-1 fluorescent antibody testing were indicative of BHV-1 infection. In the present study, BHV-1 was isolated from all fetuses. In the MLV study reported by Fickens et al,<sup>9</sup> heifers were vaccinated once prior to breeding and challenged IV at 6 to 7 months of gestation. In the vaccinated group, 84.2% were protected against abortion, whereas 100% of control heifers had fetal loss. Bovine herpesvirus type 1 fetal infection was confirmed via histologic examination, virus isolation, or both. Thus, results of those 2 MLV studies were comparable to results of the present study. In addition, the same challenge strain and model were used; however, the present study used an inactivated vaccine.

Results of the only published BHV-1 fetal loss study<sup>10,11</sup> that used an inactivated BHV-1 vaccine are difficult to compare with those of our study because of differences in study design. In the previous study, seropositive and seronegative pregnant cattle were vaccinated SC between 5 and 6 months of gestation. Cattle were challenged intratracheally or IV at approximately 7 months of gestation. Twenty-one days following challenge, the fetuses from 3 vaccinated cows (2 challenged intratracheally and 1 challenged IV) and fetuses from 2 control cows (1 challenged intratracheally and 1 challenged IV) were obtained by Caesarian section and tested for BHV-1. Fetuses removed from the 2 intratracheally challenged vaccinated cows yielded negative results for BHV-1, whereas the fetus from the IV challenged vaccinated cow yielded positive results. Both of the control fetuses yielded positive results for BHV-1. This indicates the importance of the route of challenge for evaluating BHV-1-induced fetal loss. All remaining vaccinated cattle (27/27) had normal full-term calves, whereas 4 of 8 control cattle had BHV-1 fetal loss as confirmed via virus isolation. Our study differed in that heifers were vaccinated prior to breeding; however, they were challenged at approximately 6 months of gestation, indicating vaccine duration of

at least 230 days, compared with just 21 days in the previous study. In addition, our study used only the IV challenge method, and vaccination protected 85.7% of the fetuses in the vaccinated group, whereas the other study had only 1 vaccinated animal challenged IV and that animal aborted.

Results of the present study provided evidence that an inactivated BHV-1 vaccine can provide excellent protection against fetal loss from BHV-1 infections. This is compelling evidence against the common misconception that only MLV vaccines can provide protection against BHV-1.<sup>12</sup> The protection generated by this inactivated BHV-1 vaccine can be provided without any danger of the possible abortifacient properties of the modified-live BHV-1 vaccines.

- a. ViraShield 6, Novartis Animal Health, Larchwood, Iowa.
- b. EAZI-BREED CIDR implants, Pharmacia & Upjohn Co, Kalamazoo, Mich.
- c. OvaCyst, Phoenix Scientific Inc, St Joseph, Mo.
- d. KaMaR Heatmount detectors, KAMAR Inc, Steamboat Springs, Colo.
- e. ProstaMate, Phoenix Scientific Inc, St Joseph, Mo.
- f. ViraShield 5, Novartis Animal Health, Larchwood, Iowa.
- g. National Veterinary Services Laboratory, Ames, Iowa.
- h. GLA Agriculture Electronics, San Luis Obispo, Calif.
- i. Veterinary Genetics Laboratory, University of California, Davis, Calif.
- j. Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD.
- k. Rural Technologies Inc, Brookings, SD.

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