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Protection against bovine herpesvirus type 1 (BHV-1) abortion following challenge 8 months or approximately 1 year after vaccination

Alicia D. Zimmerman,[1] *DVM*; **Angela L. Klein,**[1] *BS, RVT*; **Robin E. Buterbaugh,**[1] *MS*; **Carol L. Rinehart,**[2] *MS, PhD*; **Christopher C. L. Chase,**[1,3] *DVM, PhD, DACVM*
[1]*Rural Technologies, Inc., 1008 32nd Ave, Brookings, SD 57006*
[2]*Boehringer Ingelheim Vetmedica Inc., 2621 North Belt Highway, St. Joseph, MO 64506*
[3]*Department of Veterinary and Biomedical Science, South Dakota State University, Brookings, SD 57007*
Corresponding author: Dr. Zimmerman, t.zimmerman@hotmail.com

Abstract

Bovine herpesvirus type 1 (BHV-1) is a major cause of reproductive failure. A study was conducted to determine if vaccination with a modified-live virus (MLV) vaccine containing BHV-1 at either 8 or 13 months prior to challenge protects against BHV-1 challenge-induced abortion. A total of 51 beef heifers, seronegative to bovine viral diarrhea virus types 1 and 2 and BHV-1, were vaccinated subcutaneously with a commercially available combination MLV vaccine containing BHV-1 and inactivated bacterin vaccine, or placebo containing bacterin only. The estrus cycle was synchronized and heifers were artificially inseminated approximately 6 or 1 month(s) after vaccination. Heifers were challenge-inoculated intravenously at approximately 193 days of gestation with a virulent BHV-1 virus. Clinical signs of BHV-1 infection were monitored for 14 days following challenge. Serological status and occurrence of abortion or stillbirth were also determined. Tissues collected from aborted fetuses (n = 22) and full-term calves that were born dead (i.e., stillbirth [n = 1] or dystocia [n = 2]) were tested for BHV-1 via virus isolation. BHV-1 was isolated from 1 fetus (7.7%) from Treatment Group 1 heifers (TG1 - heifers challenged 13 months after BHV-1 MLV vaccination), from 3 fetuses/calves (15.8%) from Treatment Group 2 heifers (TG2 - heifers challenged 8 months after BHV-1 MLV vaccination), and from 7 fetuses (36.8%) from Treatment Group 3 heifers (TG3 - control heifers that received bacterin only). Polymerase chain reaction testing results indicated that 1 fetus from the 13 (7.7%) TG1 heifers, 5 fetuses out of the 19 (26.3%) TG2 heifers, and 18 fetuses out of the 19 (94.7%) TG3 control heifers were BHV-1 positive. In this study, a combination MLV vaccine containing BHV-1 administered 8 or 13 months prior to challenge provided a significant level of protection against fetal infection in the face of a substantial challenge infection with BHV-1 when compared to non-vaccinated controls.

Key words: bovine, abortion, BHV-1, vaccine

Résumé

L'herpès-virus bovin 1 (BHV-1) est une cause majeure d'insuccès à la reproduction. Une étude a été menée pour déterminer si la vaccination avec un vaccin combiné à virus vivants modifiés contenant le BHV-1 soit 8 ou soit 13 mois avant l'infection expérimentale protègeait contre l'avortement induit par l'infection au BHV-1. Un total de 51 taures de boucherie, toutes séronégatives au virus de la diarrhée virale bovine du type 1 et du type 2 et au BHV-1, ont été vaccinées par voie sous-cutanée soit avec un vaccin commercial à virus vivants modifiés contenant le BHV-1 et une bactérine inactivée ou soit avec un vaccin contenant la bactérine seule. Le cycle œstral a été synchronisé et les taures ont été inséminées artificiellement approximativement 6 ou 1 mois suivant la vaccination. Les taures ont été infectées expérimentalement par inoculation intraveineuse d'un virus virulent de BHV-1 après approximativement 193 jours en gestation. La présence de signes cliniques reliés à l'infection avec le BHV-1 a été notée pendant une période de 14 jours suivant l'infection expérimentale. On a aussi déterminé le statut sérologique et la survenance d'avortement ou de mort-nés. Les tissus recueillis de fœtus avortés (n = 22) ou de veaux morts à la naissance (i.e. mort-né [n = 1] ou dystocie [n = 2]) ont été testés pour la présence du BHV-1 par isolation virale. Le virus BHV-1 a été isolé dans les tissus d'un fœtus (7.7%) des taures du groupe de traitement 1 (taures infectées expérimentalement 13 mois après la vaccination avec le vaccin combiné), dans les tissus de trois fœtus/veaux (15.8%) des taures du groupe de traitement 2 (taures

infectées expérimentalement 8 mois après la vaccination avec le vaccin combiné) et dans les tissus de sept fœtus (36.8%) des taures du groupe de traitement 3 (taures témoins ne recevant que la bactérine). Le test de la réaction en chaîne de la polymérase a confirmé la présence de BHV-1 chez un fœtus (7.7%) des 13 taures du groupe de traitement 1, chez 5 fœtus (26.3%) des 19 taures du groupe de traitement 2 et chez 18 fœtus (94.7%) des 19 taures du groupe de traitement 3. Dans cette étude, la vaccination avec un vaccin combiné à virus vivants modifiés contenant le BHV-1 8 ou 13 mois avant l'infection expérimentale donnait un niveau de protection significativement plus grand contre l'infection fœtale après une infection substantielle avec le BHV-1 que la vaccination avec une simple bactérine inactivée.

Introduction

Bovine herpesvirus type 1 (BHV-1) is a clinically and economically significant virus of cattle, affecting operations around the world.[1,4,15,24] BHV-1 infections result in losses from animal death, abortions, and decreased production (i.e., decrease in milk production and/or weight loss),[4,23] as well as transient interference with breeding efficiency.[3,6,7,12,17] BHV-1 is the etiologic agent for the disease known as infectious bovine rhinotracheitis (IBR), or "red nose," and is associated with a variety of clinical signs, including respiratory as well as reproductive disease.[9,12,13,18,24] The virus, often linked to the bovine respiratory disease complex, can also predispose animals to secondary bacterial infections, such as pneumonia.[11,13,15,23,24] Additionally, BHV-1 is the cause of a syndrome known as infectious pustular vulvovaginitis (IPV) in heifers or infectious balanoposthitis (IBP) in bulls.[1,6,7,9,12,13,23] The causative virus for IPV/IBP (BHV-1.2) is antigenically and biologically similar to the IBR (BHV-1.1) virus;[11,12] however, it is not considered to be as highly virulent, and abortions are not commonly associated with this syndrome.[1,7] BHV-1.2 has been further subdivided into the subtypes 2a and 2b, of which only the 2a subtype has been found to be abortifacient.[12,14]

Bovine herpesvirus type 1 spreads rapidly through nasal secretions, droplets, genital secretions, serum, and fetal fluids.[9,11,14,18,22,23,24] An additional feature of the virus is the ability to become latent following infection.[1,3,7,9,11,13,14,18,23] Latent carriers serve as reservoirs for the disease, which may recur spontaneously or as a result of natural or artificial stimuli, including transport, parturition,[7,9,13,23] superinfection with another disease-causing agent, treatment with 3-methylindole,[9,23] and immunosuppressive treatment with glucocorticoids.[7,9,23]

Pregnant cattle that have not been vaccinated are susceptible to the reproductive effects of BHV-1, and infections can result in abortion rates as high as 25%.[3,6,24] Abortions resulting from infections with BHV-1 usually occur with little or no forewarning during the last half of gestation,[4] usually between 4 and 9 months gestation,[7,13,18] and up to 100[3,24] or 125[6] days after infection. The virus is considered to be ubiquitous in the United States, and as a result, most cattle have a high probability of exposure during their lifetime.[9,11] A survey conducted in the northern plains of the US found BHV-1 to be the cause of abortion in 16% of cases.[7]

Vaccination of dams is considered an effective method of controlling the spread of BHV-1. Currently, dams may be vaccinated prior to breeding with a modified-live virus (MLV) vaccine[21] or inactivated vaccine.[24] Alternatively, dams may be vaccinated during gestation using an inactivated vaccine, intranasal MLV vaccine,[9,21] temperature-sensitive MLV vaccine,[10] or MLV vaccine (in previously vaccinated animals).[5]

MLV vaccines have been shown to be effective in protecting dams from exhibiting disease and/or fetuses from becoming infected *in utero*. Out of the 7 reported fetal protection studies utilizing MLV vaccines,[4,5,6,10,17,19,20] only 2[6,20] have indicated a duration of immunity greater than 315 days. The objective of this study was to evaluate duration of immunity of the BHV-1 fraction of bovine rhinotracheitis-virus diarrhea-parainfluenza$_3$-respiratory syncytial virus vaccine (MLV), *Campylobacter fetus-Leptospira canicola-grippotyphosa-hardjo-icterohemorrhagiae-pomona* bacterin in naïve heifers vaccinated once, at approximately 12 to 14 or 17 to 19 months of age, prior to breeding and challenged with virulent BHV-1 8 or 13 months later.

Materials and Methods

Animals

Protocols were reviewed and approved by the Rural Technologies, Incorporated Institutional Animal Care and Use Committee prior to study initiation. Heifers were randomly assigned to 1 of 3 treatment groups for vaccination, and 51 of the Angus-cross beef heifers acquired for the study were subsequently challenged. Heifers in Treatment Group 1 (TG1; n = 13) received test vaccine[a] at the first vaccination (i.e., 13 months prior to challenge; 12 to 14 months of age) and placebo vaccine at the second vaccination; heifers in Treatment Group 2 (TG2; n = 19) received placebo at the first vaccination and test vaccine[a] at the second vaccination (i.e., 8 months prior to challenge; 17 to 19 months of age); and heifers in Treatment Group 3 (TG3; n = 19) received placebo vaccine at both vaccination time points. Vaccinated heifers were separated from placebo heifers at the time of each vaccination to avoid any potential vaccine virus exposures (i.e., TG1 separated from TG2 and TG3 for a period of 25 days after initial vaccination, and TG2 separated from TG1 and TG3 for a period of 27 days after second vaccination), after which the placebo and

vaccinated heifers were commingled for the remainder of the study. All heifers were managed according to routine animal husbandry procedures and were isolated from any other cattle.

Pre-vaccination Serologic Assays

Blood was collected from all heifers prior to vaccination. All heifers were seronegative for antibodies against BVDV1 and BVDV2, as well as BHV-1,[b] and were negative for BVDV by ear notch via immunohistochemical (IHC) testing.[c] Serum samples were tested for BHV-1 (Cooper's strain),[d] BVDV1 (Singer),[d] and BVDV2 (A125)[d] serum neutralizing (SN) antibody titers by use of the constant virus decreasing serum assay. Briefly, 2-fold serial dilutions (range, 1:2 to 1:256) of sera in duplicate were incubated with a constant viral titer (< 500 $TCID_{50}$) before inoculation of BVDV-free bovine turbinate cells[e] in microtiter tissue culture plates.[f] Plates were incubated at 98.6°F (37°C) with 5% CO_2 for 3 (BHV-1) to 5 (BVDV) days before being evaluated for virus-induced cytopathic effect (CPE) for BHV-1, BVDV1, and for IHC staining[2] for BVDV2. The reciprocal of the last dilution that prevented CPE formation or virus-specific staining was designated the serum neutralizing antibody titer. Geometric mean values were calculated by use of $\log_2$ titers.

Vaccination

Heifers in TG1 (n = 13) and TG2 (n = 19) were vaccinated once subcutaneously (SC) with the same serial of a commercially available MLV combination vaccine[a] containing BHV-1 at minimum immunizing dose (MID), as well as BVDV, parainfluenza 3 (PI3), bovine respiratory syncytial virus (BRSV), *C. fetus*, *L. canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohemorrhagiae*, and *L. pomona* at release levels or higher, according to manufacturer's recommendations, approximately 13 or 8 months prior to challenge, respectively. The remaining 19 control heifers were sham vaccinated twice SC (at each vaccination) with the inactivated bacterin components of the same vaccine (did not contain the viral antigens). Heifers were observed daily for 7 days after each vaccination for adverse events.

Synchronization and Breeding

At approximately 6 and 1 month(s) (TG1 and TG2, respectively) following vaccination, the heifers' estrus cycles were synchronized with a vaginal implant[g] containing progesterone, as well as injections of gonadotropin releasing hormone[i] (GnRH) and prostaglandin.[8,16] Briefly, heifers were administered prostaglandin (dinoprost tromethamine)[h] intramuscularly (IM) followed by vaginal implant insertion and GnRH administration IM 3 days following prostaglandin injection. Six days later, the implants were removed, estrus detection devices[j] were placed on the tailhead area, and the heifers were administered prostaglandin IM. Heifers were artificially inseminated twice at approximately 60 and 72 hours after implant removal with semen that was PCR[k] negative for BHV-1 and BVDV. At initial breeding, an additional dose of GnRH was administered IM if the heat detector was not activated or missing. Two, 2-year old virgin bulls vaccinated against BHV-1 and BVDV (at 4 and 7 months of age with an inactivated vaccine and at 12 months with a MLV vaccine) and negative to BVDV by ear notch testing via IHC[k] were used for pasture breeding with the heifers for 2 weeks following artificial insemination. Heifers were ultrasounded transrectally prior to challenge to confirm pregnancy status.

Challenge Inoculation

All heifers were challenged intravenously (IV) with 2 mL of BHV-1 (Cooper's strain,[l] approx 1 x 10^6 $TCID_{50}$/mL) at approximately 193 days of gestation (386 days post-vaccination [DPV] for TG1 and 233 DPV for TG2).

Post-challenge Observations

Clinical observations were performed daily by personnel that were blinded from treatment group assignment, beginning 2 days prior to challenge and continuing through day 14 after challenge. Each heifer was visually examined in the pen prior to handling and scored for clinical signs including anorexia, depression, increased respiration, coughing, nasal discharge, and ocular discharge. After the visual assessment, heifers were restrained in a standard cattle chute to measure body temperatures using a rectal thermometer.[m] Additionally, 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 (0 and 153 DPV), prior to breeding (181 DPV), 3 months following breeding (274 DPV), 1 day prior to challenge (385 DPV), 2 weeks after challenge (400 DPV), and following abortion (as appropriate). Blood was processed for serum and utilized to determine antibody titers against BHV-1 via SN, as described above.

Neonatal calves were tested for antibody to BHV-1. Calves were bled prior to colostrum ingestion, and SN antibody titers against BHV-1 and BVDV were determined via the constant virus-decreasing serum assay, as described above. Serum samples from calves were also tested for gamma-glutamyl transferase (GGT)[k] to determine if calves had suckled prior to blood collection.

Fetal Tissue Collection and Testing

Samples were collected from aborted fetuses and calves that died during parturition (i.e., dystocia) and were tested for BHV-1. Thymus, lung, liver, kidney,

and brain (cerebellum) were each tested for BHV-1 and BVDV via virus isolation.[b] Briefly, dilutions of processed samples were made and each diluted sample was added in triplicate to BVDV-free bovine turbinate cell monolayers in microtiter tissue culture plates. The culture plates were incubated for 2 to 3 days for BHV-1 and 4 to 5 days for BVDV at 98.6°F (37°C) with 5% CO_2. Results were considered positive if virus-specific staining was observed in inoculated cells. The same fetal tissues tested for VI were used to test for BHV-1 using a PCR[k] assay. Heart blood, pleural fluid, or both were tested for neutralizing antibody titers against BHV-1. In conjunction with viral testing, fetal spleen samples were matched to the appropriate dam (using tail-switch hair samples) via deoxyribonucleic acid (DNA) parentage testing.[n] Lung, placenta, and stomach contents were tested for abortagenic bacteria, and a kidney sample was tested for leptospiral organisms via fluorescent antibody testing.[k]

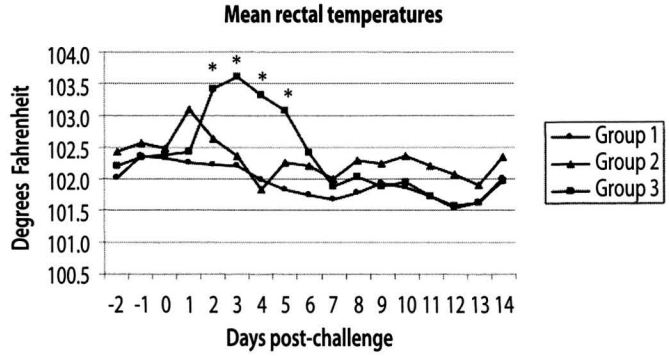
Statistical Analyses

Statistical analysis was done with SAS© Version 9.2,[o] and the significance of differences between each treatment group and the control group was set at α-level 0.05. Binomial type variables (abortion, cough, diarrhea, pyrexia, virus isolation, and PCR) were analyzed with the FREQ procedure and Fisher's Exact Test. The NPAR1WAY procedure and Wilcoxon Rank Sum Test were utilized for the analyses of number of days with cough, diarrhea, and pyrexia. The MIXED procedure was utilized for the repeated measures analyses of rectal temperature and for SN titer. The model included the fixed effects of group, day, and group-by-day interaction and the random effects between and within animals with a compound symmetry covariance structure.

Results

Clinical Observations

No clinical signs or adverse vaccine reactions were observed in any heifers following each vaccination (data not shown). Following challenge, body temperature was measured rectally in all heifers every day starting 2 days prior to challenge through 14 days after challenge, with the mean determined for each group (Figure 1). Heifers in TG1 and TG2 had significantly ($P \leq 0.0002$) lower rectal temperatures on 2, 3, 4, and 5 days post-challenge (DPC) than heifers in TG3. Mean rectal temperatures for TG1 were 102.2°F (39°C), 102.2°F (39°C), 102.0°F (38.9°C), and 101.8°F (38.8°C), and 102.6°F (39.2°C), 102.4°F (39.1°C), 101.8°F (38.8°C), and 102.3°F (39.1°C) for TG2 on 2, 3, 4, and 5 DPC, respectively; whereas, the controls had mean rectal temperatures of 103.4°F (39.7°C), 103.6°F (39.8°C), 103.3°F (39.6°C), and 103.1°F



*Indicates significant and clinically relevant differences between Group 1 and 2 (vaccinate groups) versus Group 3 (control group).

Figure 1. Mean rectal temperatures for all treatment groups before and after challenge with virulent BHV-1. Heifers in Treatment Group 3 (black squares; n = 19) had significantly ($P \leq 0.0002$) higher mean rectal temperatures than heifers in Treatment Groups 1 (black circles; n = 13) and 2 (black triangles, n = 19) on 2 to 5 DPC, which was clinically relevant. Various significant differences were also noted on days 1, 6, 8, and 10 to 12 DPC; however, the difference was not clinically significant (i.e., no febrile response).

(39.5°C) on 2, 3, 4, and 5 DPC (Figure 1). The difference noted is also clinically relevant, as the mean rectal temperatures for TG1 and TG2 were within normal limits, while the mean rectal temperatures for TG3 were elevated above 103.0°F (39.4°C). Specifically, 9, 3, and 1 heifer(s) in TGs 3, 2, and 1, respectively, had rectal temperatures ≥ 103.5°F (39.7°C) on 2 DPC; 13 heifers in TG3 and 1 heifer each in TGs 1 and 2 had rectal temperatures ≥ 103.5°F (39.7°C) on 3 DPC; 10 heifers in TG3 and 1 heifer in TG1 had rectal temperatures ≥ 103.5°F (39.7°C) on 4 DPC; and 7 heifers in TG3 and no heifers in TGs 1 and 2 had rectal temperatures ≥ 103.5°F (39.7°C) on 5 DPC. Interestingly, on 1 DPC, 4 heifers in TG2 had rectal temperatures ≥ 103.5°F (39.7°C; mean of 103.1°F [39.5°C]), versus 0 heifers in TG3 (mean of 102.4°F [39.1°C]) and 1 heifer in TG1 (mean of 102.3°F [39.1°C]), which was significant ($P \leq 0.0014$). Additionally, various statistically significant differences were noted as TG2 had significantly higher ($P \leq 0.0444$) mean rectal temperatures than TG1 and TG3 on 11 and 12 DPC; TG1 had significantly lower ($P \leq 0.0480$) mean rectal temperatures than TG2 and TG3 on 6 DPC; and TG1 had significantly lower ($P = 0.0273$ and $P = 0.0366$) mean rectal temperatures than TG2 on 8 and 10 DPC, respectively; however, the differences were not clinically relevant, as the rectal temperatures were not elevated above normal limits. Following challenge, there were

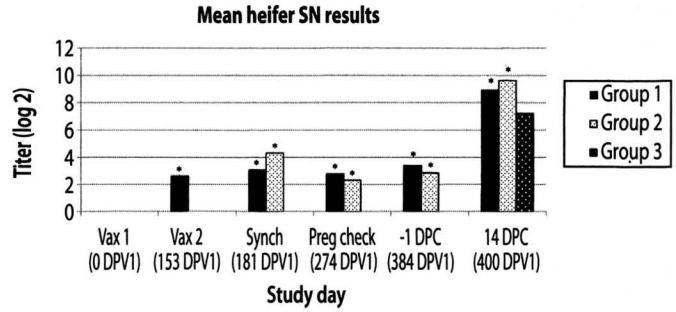
mild clinical signs in all treatment groups (i.e., cough and diarrhea), which was not significant between groups (data not shown).

A total of 26 heifers delivered live calves (11 heifers in TG1 [84.6%], 14 heifers in TG2 [73.7%], and 1 heifer in TG3 [5.3%]); whereas, 22 heifers (1 heifer in TG1 [7.7%], 3 heifers in TG2 [15.8%], and 18 heifers in TG3 [94.7%]) aborted between 16 to 46 days following challenge. Three additional heifers either delivered a stillborn calf (1 heifer from TG2), or required assistance in delivery (i.e., dystocia) and delivered dead calves (1 heifer from TG1 and 1 from TG2).

Serum Neutralizing Antibody Titers

All heifers were seronegative against BHV-1 at the time of the first vaccination (TG1 heifers vaccinated) (Figure 2). The remaining animals in TG2 and TG3 were seronegative for BHV-1 at the time of second vaccination (vaccination of TG2 heifers only); whereas, TG1 heifers had a significantly higher ($P < 0.0001$) mean titer of 2.62_{log2} (specifically, 11 out of 13 heifers were seropositive [titer range of $1.00 - 5.00_{log2}$]). At the time of initiation of estrus synchronization (181 days following vaccination of TG1 heifers and 28 days following vaccination of TG2 heifers), mean titers were significantly higher ($P < 0.0001$) in both groups of vaccinated heifers (3.08_{log2} for TG1 and 4.32_{log2} for TG2) when compared to controls (TG3) (Figure 2). Specifically, 11 of 13 TG1 heifers were seropositive (titer range of $2.00 - 5.00_{log2}$), and 19 of 19 TG2 heifers were seropositive (titer range of $1.00 - 6.00_{log2}$), while all 19 heifers in TG3 were seronegative. At approximately 81 days of gestation (274 days following TG1 vaccination and 121 days following TG2 vaccination), mean titers were significantly higher ($P < 0.0001$) in both groups of vaccinates (2.77_{log2} and 2.32_{log2} for TG1 and TG2, respectively) when compared to TG3. Specifically, 11 of 13 heifers in TG1 were seropositive (titer range of $2.00_{log2} - 6.00_{log2}$) and 17 of 19 heifers in TG2 were seropositive (titer range of $1.00_{log2} - 4.00_{log2}$); whereas, the control heifers (TG3) remained seronegative (Figure 2). On the day prior to challenge, vaccinated heifers in TG1 (385 days following vaccination) had a significantly higher ($P < 0.0001$) mean titer of 3.38_{log2} and vaccinated heifers in TG2 (232 days following vaccination) had a significantly higher ($P < 0.0001$) mean titer of 2.84_{log2} compared to TG3 heifers. Specifically, 11 of 13 TG1 heifers were seropositive (titer range of $2.00 - 6.00_{log2}$) and 17 of 19 heifers were seropositive (titer range of $1.00 - 5.00_{log2}$) against BHV-1; whereas, control heifers (TG3) again remained seronegative. At the conclusion of the challenge portion of the study (14 DPC), vaccinated heifers in TG1 (400 days following vaccination) had a significantly higher ($P < 0.0001$) mean titer of 8.92_{log2} and vaccinated heifers in TG2 (247 days following vaccination) had a significantly higher ($P < 0.0001$) mean titer of 9.63_{log2} when compared to placebo-vaccinated heifers in TG3 (mean titer of 7.21_{log2}). Specifically, 13 of 13 TG1 heifers were seropositive (titer range of $7.00 - 10.00_{log2}$), 19 of 19 TG2 heifers were seropositive (titer range of $9.00 - 11.00_{log2}$), and 19 of 19 TG3 heifers were seropositive (titer range of $5.00 - 9.00_{log2}$) against BHV-1 (Figure 2). Additionally, blood was collected from the 22 heifers (1 heifer from TG1, 3 heifers from TG2, and 18 heifers from TG3) that were determined to have aborted, which does not include the 2 heifers (1 from TG1 and 1 from TG2) with calves that were determined to have died as a result of dystocia and 1 TG2 heifer that delivered a stillborn calf. Specifically, the TG1 heifer had a titer of 5.00_{log2}, the 3 TG2 heifers had a titer range of $5.00 - 7.00_{log2}$, and the 18 TG3 heifers had a titer range of $5.00 - 8.00_{log2}$.

Twenty-seven calves were born from 26 heifers (i.e., 1 set of twins) and observed as normal post-calving.



*Indicates significant difference between Group 1 and/or 2 (vaccinate groups) versus Group 3 (control group).

Figure 2. Mean heifer serology results against BHV-1 before and after vaccinations 1 (0 DPV) and 2 (153 DPV), at estrus synchronization (181 DPV), at pregnancy check (274 DPV), on the day prior to challenge (-1 DPC) with virulent BHV-1, and at the end of the clinical observations period (14 DPC). All heifers were seronegative at the time of first vaccination. Heifers in Treatment Group 1 (solid black column; n = 13 [i.e., heifers vaccinated at the time of first vaccination]) had developed mean titers against BHV-1 on 153 DPV, which was significantly different from Treatment Groups 2 (white column with back dots; n = 19 [i.e., heifers vaccinated at the time of second vaccination; 153 DPV]) and 3 (gray column with white dots; n = 19 [i.e., control heifers]) ($P < 0.0001$). On 181 DPV, 274 DPV, and 0 DPC, heifers in Treatment Groups 1 and 2 had significantly higher titers ($P < 0.0001$) than heifers in Treatment Group 3. Heifers in Treatment Group 3 remained negative until after challenge. Notice that on 14 DPC heifers in Treatment Group 3 had significantly lower titers ($P < 0.0001$) than heifers in Treatment Groups 1 and 2 (challenged 13 and 8 months after vaccination, respectively).

Six of the calves had BHV-1 titers of 4.00_{log2} to 6.00_{log2} at birth; however, 5 of those calves were GGT positive (i.e., values of > 51 U/L), indicating that they had suckled prior to blood collection (Table 1). Further, of the set of twins (TG2), 1 calf had a BHV-1 titer (4.00_{log2}) and a concurrent GGT value of 56 U/L, while the other calf was seronegative to BHV-1 and had a GGT value of 10 U/L. This indicates the calves that suckled (i.e., GGT positive) were likely BHV-1 positive as a result of successful passive transfer of antibody from the dams. Consequently, only 1 of the calves from the vaccinated heifers (from TG1) could be definitively shown to have been exposed to BHV-1 in utero (i.e., had a positive titer [5_{log2}] and was GGT negative [GGT value of 14 U/L]). The 1 live calf born to a control heifer (from TG3) was serologically negative prior to colostrum ingestion (Table 1). The remaining 21 calves from vaccinated heifers had BHV-1 titers of $\leq 1_{log2}$ at birth.

Fetal Tissue Results

A total of 25 heifers aborted (n = 22) or experienced late-term fetal loss (n = 3). Mean DPC to abortion for fetuses determined to be positive for BHV-1 was 46, 48.8, and 21.7 in TGs 1, 2, and 3, respectively (Table 2). BHV-1 virus was isolated from 11 fetuses/calves (1 of 13 in TG1 [7.7%], 3 of 19 in TG2 [15.8%], and 7 of 19 in TG3 [36.8%]) using VI (Table 2), and the proportion positive for all groups did not differ significantly ($P \geq 0.1006$). Conversely, the number of fetuses/calves positive for BHV-1 via PCR was significantly higher ($P < 0.0001$) in TG3 (18 of 19 [94.7%]) when compared to TG1 (1 of 13 [7.7%]) and TG2 (5 of 19 [26.3%]) (Table 2); however, the number of fetuses/calves positive for BHV-1 via PCR did not differ ($P = 0.3606$) between TG1 and TG2. Specifically, all aborted fetuses were determined to be BHV-1 positive via PCR testing. Additionally, the stillborn calf and the calf that died as a result of dystocia (both from TG 2) were found to be positive for BHV-1 via PCR testing, indicating that had these calves been born live, they would likely have been infected, while tissues from the calf of the TG1 heifer with dystocia were negative for BHV-1 via VI and PCR testing (Table 2).

Titers against BHV-1 in pleural fluid and heart blood samples from all aborted fetuses/calves were determined to be $< 4.00_{log2}$, as titers could not be determined at lower dilutions due to the technical difficulties of evaluating these fetal fluid samples (i.e., hemolyzed fluids obscuring visualization of CPE in cell monolayers). Specifically, the heart blood samples from the fetuses in TG1 had a mean titer of 2.5_{log2}, including the calf that died as a result of dystocia (titer of 3_{log2}); titers from fetuses in TG2 ranged from $2_{log} - 4_{log2}$, with a mean of $3_{log2,}$ including the calf that died as a result of dystocia (titer of 3_{log2}) and the stillborn calf (titer of 4_{log2}); and the fetuses in TG3 ranged from 1_{log2} (n = 1) to 4_{log2} (n = 1), with a mean of 2.22_{log2}. Titers from 24 of the pleural fluid samples were $< 2.00_{log2}$ and 1 sample (TG3) was $< 1.00_{log2}$.

Table 1. Calf antibody titer results against BHV-1 after birth and before colostrum ingestion following challenge of dams with virulent BHV-1. Out of all live calves born of dams in Treatment Groups 1 (n = 11) and 2 (n = 15; including 1 set of twins), 2 and 3 calves, respectively, had pre-colostral antibody titers against BHV-1; however, 1 calf from TG1 and all calves from TG2 also had GGT levels of >51 U/L. One live calf was born of a dam in Treatment Group 3, and did not have a pre-colostral antibody titer against BHV-1.

Treatment group	Number positive†	SN / GGT positive	Live calves born
1	1	3/2	11
2	0	3/3	15
3	0	0/0	1

†Excludes calves with elevated GGT (2 from TG1; 3 from TG2).

Table 2. Results from VI and PCR testing of fetal tissues (i.e., liver, lung, kidney, brain, and thymus) against BHV-1 following abortion after challenge with virulent BHV-1. Out of a total of 2 heifers that aborted in Treatment Group 1, 1 fetus was negative and 1 fetus was positive for BHV-1 via VI and PCR (in all tissues). All fetuses (n = 5) aborted from heifers in Treatment Group 2 were positive for BHV-1 via PCR in all tissues collected; however, only 3 were positive via VI testing. All fetuses (n = 18) aborted from heifers in Treatment Group 3 were positive for BHV-1 via PCR in all tissues collected; however, only 7 were positive via VI testing.

Treatment group	Total number aborted*†	Fetal tissue results* VI positive	PCR positive**	PCR negative
1	2/13[a]	1	1	1
2	5/19[a,b]	3	5	0
3	18/19	7	18+	0

*Includes 2 calves that died as a result of dystocia[a] (i.e., 1 each from TG1 and TG2) and 1 stillborn[b] calf.
†Total number aborted out of total number in Treatment Groups.
**Positive fetuses = positive in all tissues tested (i.e., liver, lung, kidney, brain, and thymus).
+Indicates significant difference between TG3 (control group) versus TG1 and TG2 (vaccinate groups).

All 25 fetuses/calves were negative for BVD (via VI), aerobic bacteria (lung and stomach contents), and leptospires (via fluorescent antibody [FA] testing).[k] Two heifers had bacteria isolated from the placenta (*Escherichia coli* from 1 heifer in TG3 and *Campylobacter sp* from 1 heifer in TG2);[k] however, since the placentas were often collected directly from the ground, the isolates were determined to be a result of environmental contamination, rather than a pathogen that caused the abortion/stillbirth.

Discussion

This study demonstrated that a single dose of a multivalent MLV vaccine[a] containing BHV-1 at MID, as well as BVDV, PI3, BRSV, *C. fetus*, *L. canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohemorrhagiae*, and *L. pomona* at release levels or higher, administered approximately 13 and 8 months prior to challenge, prevented abortion despite a virulent BHV-1 challenge at approximately 193 days of gestation (i.e., 386 DPV for TG1 and 233 DPV for TG2). Vaccinated heifers had significantly lower rectal temperatures (on 2 to 5 DPC) and significantly fewer abortions. Vaccinated heifers in TG1 and TG2 had mean rectal temperatures of 101.9°F (38.8°C) and 102.3°F (39.1°C), respectively. The mean rectal temperature of control heifers was also 102.3°F (39.1°C); however, the heifers in this treatment group exhibited pyrexia on 4 days (i.e., mean rectal temperatures of 103.4°F [39.7°C], 103.6°F [39.8°C], 103.3°F [39.6°C], and 103.1°F [39.5°C] on 2, 3, 4, and 5 DPC, respectively [at the peak of clinical signs]) whereas heifers in TG2 only exhibited elevated rectal temperatures on day 1 (mean rectal temperature of 103.1°F [39.5°C] on 1 DPC), and mean rectal temperatures were within normal limits on 2, 3, 4, and 5 DPC (i.e., 102.6°F [39.2°C], 102.4°F [39.1°C], 101.8°F [38.8°C], and 102.3°F [39.1°C], respectively). Bovine herpesvirus type 1 was detected via PCR in all 22 aborted fetuses, 1 stillborn calf (TG2), and 1 calf that died as a result of dystocia (TG2). No other abortifacient pathogens were identified. Among vaccinated heifers in TG1 and TG2, 92.4% (12/13) and 73.7% (14/19), respectively, were protected against BHV-1-induced abortion, whereas 94.7% (18/19) of fetuses from control heifers were BHV-1 infected following challenge, as determined by PCR.

Vaccination with a MLV vaccine is a beneficial practice in breeding females for a variety of reasons. The ability of a MLV vaccine to protect dams from abortion is important, because often abortions occur without clinical signs of disease.[4,9,13,17,22] The present study demonstrated that a MLV vaccine administered approximately 1 year prior to challenge effectively protects from BHV-1-induced fetal infection and subsequent abortion. While the present study did not involve vaccination of pregnant animals, this vaccine, among others, may be administered to pregnant cattle where indicated in label directions. It is important to note, however, that abortions can occur when MLV vaccines are administered to animals with unknown or questionable vaccine status.[5,10,17] Additionally, MLV vaccines have also been shown to be effective at preventing IPV/IPB,[23] which can inhibit reproductive efficiency.[12]

Previously published BHV-1 studies that examine fetal protection have been performed with MLV vaccines.[4,5,6,10,17,19,20] Since challenge models and study designs are not consistent among studies, it is difficult to compare results from those studies with the present study. Two studies used 2 doses of a MLV vaccine administered IM prior to breeding, followed by an IV challenge with virulent BHV-1. These studies showed 90% protection from abortion in vaccinated animals versus a 100% abortion rate in control animals,[4] and 92% protection from abortion in vaccinated animals versus a 92% abortion rate in control animals.[17] Saunders et al[19] reported 94.1% protection from abortion in vaccinated animals versus a 62.5% abortion rate in control animals when heifers were vaccinated IM with a MLV vaccine once or twice prior to breeding, before IN challenge at either 3, 4.5, or 6 months of gestation, whereas the present study showed similar results (i.e., 92.3% [TG1] and 73.7% [TG2] protection from BHV-1-induced abortion [as determined via PCR] versus a 94.7% [18/19] abortion rate in control animals) with only 1 SC dose of a MLV vaccine. Kit et al[10] reported 100% prevention of abortion/fetal infection in dams vaccinated either IM or intravaginally during pregnancy with 1 dose of a thymidine kinase-negative, temperature-resistant IBR virus (i.e., BHV-1) prior to IN challenge with virulent BHV-1, versus 50% protection from fetal infection in non-vaccinated animals. The only true parallel between this study and the present study, however, is that dams that were vaccinated once were well protected from abortion and/or fetal infection.

On the other hand, 2 studies have been performed that are more similar to the present study (i.e., challenge at ≥ 316 DPV). Smith et al[20] reported a study in which heifers were vaccinated IN with a single dose of an IBR-PI3 vaccine, followed by subsequent IN challenge with virulent BHV-1 at 316 DPV (7.5 to 9 months of gestation). Vaccinated animals in this study had decreased levels of pyrexia and clinical signs following challenge when compared to non-vaccinated controls. Additionally, all vaccinated dams (15/15) gave birth to normal, BHV-1 negative calves, whereas 12 of 16 non-vaccinated controls exhibited abortion or neonatal calf death, and BHV-1 was isolated from 11 of those offspring. In contrast, no significantly elevated clinical signs (i.e., cough and diarrhea) were noted following challenge in the present study; however, vaccinates in TG1 and TG2 did have significantly lower rectal

temperatures on 2 to 5 DPC. While similar abortion rates were noted in both studies, BHV-1 was found (via PCR) in all aborted control (TG3) fetuses in the present study. In 2006, Ficken et al reported a study in which heifers were vaccinated IM with either the test vaccine (i.e., MLV containing BHV-1, BVDV1, BVDV2, PI3, and BRSV, reconstituted with an inactivated 5-way leptospira and *C. fetus* bacterin) or placebo (inactivated bacterin only) and were subsequently challenged IV at 365 DPV (between 193 and 215 days gestation).[6] In this study, 84.2% of vaccinated heifers were protected against abortion, whereas 100% of controls had fetal loss, and BHV-1 fetal infection was confirmed via histologic examination, VI, or both. Thus, results of this study were comparable to the present study; however, protection from fetal infection in the present study was higher (92.3%) in heifers challenged 20 additional days after vaccination (i.e., 385 DPV; TG1), versus a 94.7% abortion rate in control heifers (as verified by PCR). The significance of the current study is that it is the first study to demonstrate a high level of protection from BHV-1-induced abortion in heifers that have been vaccinated once SC with a MLV vaccine that provides duration of immunity greater than 1 year.

The present study offers evidence that a multivalent MLV BHV-1 vaccine is beneficial, as it offers protection against abortion from BHV-1 infections when administered approximately 13 months prior to challenge with virulent BHV-1, which allows for greater flexibility in vaccination timing. Additionally, vaccinated dams gave birth to live calves that were seronegative to BHV-1, indicating protection from fetal infection as well.

Conclusion

A vaccine that is effective in preventing abortion resulting from maternal infection with BHV-1 is an essential part of cattle production. Results from this study supported that 1 SC dose of the multivalent vaccine containing MLV BHV-1 used in this study, administered to heifers approximately 8 or 13 months prior to challenge, effectively aids in the prevention of abortion and fetal infection when challenged with virulent BHV-1. Consequently, initial vaccination with a MLV vaccine followed by yearly booster vaccinations (administered prior to or during gestation, per label directions) can provide significant protection from BHV-1 infection to a majority of cattle, and can help prevent subsequent development of latent carriers by producing seronegative calves.

Endnotes

[a]Express® FP 5-VL5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[b]Rural Technologies, Inc., Brookings, SD

[c]Nebraska Veterinary Diagnostic Laboratory, University of Nebraska-Lincoln, Lincoln, NE

[d]National Veterinary Services Laboratory, Ames, IA

[e]American Type Culture Collection, Manasses, VA

[f]Greiner Bio-One, Frickenhausen, Germany

[g]EAZI-BREED™ CIDR® implants, Pharmacia & UpJohn Company, New York, NY

[h]Lutalyse®, Pharmacia & UpJohn Company, New York, NY

[i]Cystorelin®, Merial Limited, Duluth, GA

[j]KAMAR® Heatmount®, Kamar Inc., Zionsville, IN

[k]Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD

[l]BHV-1 Cooper's Strain (Lot Number CHV OC-22 M 11-3-00 9-6-01), Rural Technologies, Inc., Brookings, SD. Strain originally obtained from the Center for Veterinary Biologicals (CVB)

[m]GLA Thermometer, GLA Agricultural Electronics, San Luis Obispo, CA

[n]Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA

[o]SAS® System, Version 9.2, SAS Institute, Cary, NC

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Injectable Baytril® 100 (enrofloxacin)

100 mg/mL Antimicrobial Injectable Solution
For Subcutaneous Use In Beef Cattle, Non-Lactating Dairy Cattle And Swine Only
Not For Use In Female Dairy Cattle 20 Months Of Age Or Older Or In Calves To Be Processed For Veal

BRIEF SUMMARY:
Before using Baytril® 100, please consult the product insert, a summary of which follows:

CAUTION:
Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.
Federal (U.S.A.) law prohibits the extra-label use of this drug in food-producing animals.

PRODUCT DESCRIPTION:
Each mL of Baytril® 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

INDICATIONS:
Cattle - Single-Dose Therapy: Baytril® 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* in beef and non-lactating dairy cattle; and for the control of BRD in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis.*
Cattle - Multiple-Day Therapy: Baytril® 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* in beef and non-lactating dairy cattle.
Swine: Baytril® 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis, Streptococcus suis, Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae.*

RESIDUE WARNINGS:
Cattle: Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. This product is not approved for female dairy cattle 20 months of age or older, including dry dairy cows. Use in these cattle may cause drug residues in milk and/or in calves born to these cows. A withdrawal period has not been established for this product in preruminating calves. Do not use in calves to be processed for veal.
Swine: Animals intended for human consumption must not be slaughtered within 5 days of receiving a single-injection dose.

HUMAN WARNINGS:
For use in animals only. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

PRECAUTIONS:
The effects of enrofloxacin on cattle or swine reproductive performance, pregnancy and lactation have not been adequately determined.
The long-term effects on articular joint cartilage have not been determined in pigs above market weight.
Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.
Baytril® 100 contains different excipients than other Baytril® products. The safety and efficacy of this formulation in species other than cattle and swine have not been determined.
Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

ADVERSE REACTIONS:
No adverse reactions were observed during clinical trials.

ANIMAL SAFETY:
In cattle safety studies, clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance and incoordination were observed when a dose of 50 mg/kg was administered for 3 days. An injection site study conducted in feeder calves demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue and underlying muscle.
In swine safety studies, incidental lameness of short duration was observed in all groups, including the saline-treated controls. Musculoskeletal stiffness was observed following the 15 and 25 mg/kg treatments with clinical signs appearing during the second week of treatment. Clinical signs of lameness improved after treatment ceased and most animals were clinically normal at necropsy. An injection site study conducted in pigs demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue.
U.S. Patent No. 5,756,506

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