PEER REVIEWED

Efficacy of a combination modified-live IBR-BVD-PI3-BRSV vaccine + *Mannheimia haemolytica* toxoid against challenge with virulent BVDV-1b and BVDV-2 viruses in young calves 60 days of age

Aleksandar Masic, DVM, PhD; **Brian Sobecki,** BSc; **Suman Mahan,** BVM, MSc, PhD; **Chadwick Brice,** BSc; **Sally Mattern,** BSc; **Dennis Peterson,** BSc; **Peter Barret,** DVM; **Colt Daugherty,** DVM, MPH; **Todd Meinert,** PhD; **Ignacio Correas,** DVM, PhD, DACVM; **Véronique Moulin,** PhD
Veterinary Medicine Research and Development, Zoetis Inc, Kalamazoo, MI 47001
Corresponding author: Dr. Aleksandar Masic; Aleksandar.Masic@zoetis.com

Abstract

Efficacy of attenuated (*att*) bovine viral diarrhea virus (types 1 and 2) as antigen fractions in a modified-live multivalent vaccine were evaluated following single, subcutaneous (SC) administration and intranasal (IN) challenge 35 to 38 d after vaccination, with either virulent BVDV-1b (Study 1) or BVDV-2 (Study 2) viruses in young calves. A total of 80 BVDV-seronegative Holstein calves, 53 to 61 d of age at the time of vaccination, were used in 2 separate studies with 40 animals per study. In each study, calves were allocated to 1 of 2 treatment groups (20 animals per group) which received either a single dose of combination modified-live bovine rhinotracheitis (BHV-1)-bovine virus diarrhea-parainfluenza 3-respiratory syncytial virus vaccine + *Mannheimia haemolytica* toxoid, or corresponding placebo formulation without targeted test antigen fractions *att*BVDV-1a and *att*BVDV-2. In the respective studies, multivalent vaccine induced significantly higher virus neutralizing antibody responses and reduced incidence and duration of leukopenia and viremia in vaccinated animals compared to placebo-treated animals. Post-challenge leukopenia, a hallmark of BVDV infection, was observed in 75% and 100% of control calves compared to only 26.3% and 25% in vaccinated animals in Study 1 and Study 2, respectively (p=0.006; p=0.0001). In addition, duration of leukopenia was significantly shorter in vaccinates compared to placebo controls (p=0.0091 Study 1; p<0.0001 Study 2). Furthermore, 100% of placebo-treated calves in both studies were viremic compared to 57.9% (Study 1) and 25% (Study 2) of vaccinated animals, resulting in significant reduction of post-challenge viremia (p=0.0012, Study 1; p=0.0001, Study 2). The duration of viremia was significantly shorter (p<0.0001) in vaccinated groups compared to control calves in both studies. In conclusion, data from the current studies demonstrated vaccine efficacy in 60-day-old calves against BVDV-1b and BVDV-2 infection.

Key words: vaccine, BVDV-1, BVDV-2, BRD, MH toxoid, leukopenia, viremia

Introduction

Bovine respiratory disease (BRD) is the most common disease in beef and dairy cattle, and a major cause of morbidity, mortality, and economic losses in the cattle industry worldwide.[17] BRD consequently results in increased use of antibiotics in feedlots, which could pose public health concerns due to increased antibiotic resistance.[27,31] The pathophysiology of BRD is complex and includes interactions between pathogen, host, genetics, environment, and management practices. BRD is commonly triggered by viral infection exacerbated by stress and followed by secondary infection with commensal bacteria.[25] Viral infection increases susceptibility and predisposes affected animals to secondary bacterial infections due to immunosuppression or excessive inflammation, resulting in damage of the epithelium of the upper and lower respiratory tract. This damage facilitates the migration of bacterial pathogens and colonization of the lower respiratory tract, consequently leading to bronchopneumonia.[2,19] Multiple viral pathogens have been implicated in BRD, including bovine viral diarrhea virus (BVDV), bovine herpesvirus type-1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenza type-3 (PI3), and more recently, bovine coronavirus (BCoV).[13,21,28]

Bovine viral diarrhea virus (BVDV) has been associated with BRD in calves of mixed source, auction market derived, commingled, transported, and those observed with BRD signs.[10,13] The broad nature of the disease, transmission, and lack of treatment have made it globally enzootic, and one of the most significant cattle diseases.[18,36]

BVDV is a small, enveloped, single-stranded positive sense RNA virus, 12.5 kb in size, and classified to the genus *Pestivirus* and *Flaviviridae* family. BVDVs can be further

© Copyright American Association of Bovine Practitioners; open access distribution.

distinguished by their biotype and genotype.[1] The biotypes, cytopathic (CP) and noncytopathic (NCP), are based on the presence or absence of visible cytopathic effects (CPE) in infected tissue cultures. BVDV genotypes (1 and 2) are differentiated from each other and from other *Pestiviruses* by monoclonal antibodies (mAbs) directed against the E2 protein, or by genetic analysis of different regions of the genome.[29,30,33] Both genotypes are further divided into subtypes, and at least 15 sub-genotypes of BVDV-1 and 2 sub-genotypes of BVDV-2 have been identified.[33] BVDV- 1 genotypes were isolated more frequently than BVDV-2 genotypes from necropsy cases of calves associated with severe BRD with fibrinous pneumonia.[14] Furthermore, BVDV-1b subtype was more often isolated than BVDV-1a (68.3% vs 31.7%) in cattle diagnosed with BRD and from calves that died due to pneumonia.[11] Although both BVDV genotypes (1 and 2) cause disease, severe cases of clinical disease are more commonly associated with the BVDV-2 genotype.[20] Animals with either BVDV-1 or BVDV-2 infection commonly develop leukopenia (neutropenia and lymphopenia) and thrombocytopenia.[5,7] These hematologic abnormalities may contribute to clinical signs and disease outcome, and could be considered as a hallmark of BVDV infection in cattle.[2] The pathogenesis of BVDV-induced leukopenia has not been fully described; however, viral antigen was observed in bone marrow myeloid cells after experimental infection with BVDV-1 and BVDV-2 which could alter bone marrow production.[35,39] Programs used to control BVDV in many countries worldwide largely depend on the detection and removal of BVDV persistently infected (PI) animals, and prevention of introduction of PI animals in the herds with biosecurity programs and vaccination.

Unvaccinated calves are highly susceptible to BVDV infections and are at the high risk of developing BRD with potentially lethal consequences. Several modified-live and killed viral and bacterial vaccines are available commercially, and their efficacy in reducing the morbidity and mortality in calves due to BRD, including BVDV-1 and BVDV-2 pathogens, has been demonstrated.[6,8,24,40,41] The timing of vaccine administration against BRD antigens is key for vaccine efficacy and BRD prevention.[32] In North America, the most common times to vaccinate cattle are at key animal handling time periods such as shortly after birth (neonatal calves), during branding (nursing calves 60 to 120 d of age), at weaning (~205 d of age), and during initial processing and entry at stocker, backgrounders, and/or feedlot facilities.[32] In the present report we share the results from 2 separate studies in which the aim was to evaluate the efficacy of *att*BVDV-1b and *att*BVDV-2 fractions from a combination multivalent, modified-live vaccine-toxoid in calves ~60 d of age following single, SC administration and challenge with virulent BVDV-1 and BVDV-2 viruses at 35 to 38 d post-vaccination.

Materials and Methods

Animals and Housing

Eighty (80) BVDV-1 and BVDV-2 serologically negative (SN titer <1:2), colostrum-deprived Holstein calves were sourced from commercial dairies on 2 occasions (40 animals each time) for the purposes of 2 separate studies (BVDV-1, Study 1; BVDV-2, Study 2). The calves were 53 to 55 (Study 1) and 54 to 61 (Study 2) d of age at the time of study start and vaccination (d 0) (Table 1). All animals were housed at a commercial farm for 25 and 29 d post-vaccination, and then shipped to the Zoetis research farm (Richland, MI) 10 d prior to the scheduled challenge phase. Newborn calves were fed electrolytes until 48 h of age to avoid ingestion of maternal antibodies, after which they received milk replacer twice a day every 12 hours until 6 weeks of age; thereafter, calves were fed with an age appropriate diet that met or exceeded nutritional requirements while water was provided *ad libitum.* At birth, calves were vaccinated against enteric rotavirus and coronavirus[a] as per manufacturer label recommendations, and no other treatments were administered. Prior to shipment to the challenge facility, all calves were administered an antibiotic.[b] During the vaccination phase, animals in both Study 1 and Study 2 were housed in individual hutches at the commercial dairy until they were transported to the challenge facility. Following arrival and post-challenge at the research

Table 1. Summary of study design in calves experimentally challenged with BVDV-1b (Study 1) or BVDV-2 (Study 2) after vaccination with a combination multivalent 5-way MLV vaccine + *Mannheimia haemolytica* toxoid.

Virus	Strain	Route of challenge	Number of calves per treatment group		Dose of challenge ($TCID_{50}$) and volume per animal	Age at vaccination (days)	Study day of	
			Vaccine†	Placebo†			Challenge	Completion
BVDV-1 (Study 1)	NY-1b	Intranasal	20	20	6.9 log_{10} 4 mL	53-55	35	49
BVDV-2 (Study 2)	#24515	Intranasal	20	20	5.4 log_{10} 5 mL	54-61	38	52

†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

© Copyright American Association of Bovine Practitioners; open access distribution.

facility, calves were commingled, allotted by treatment, and placed into 2 identical rooms in a BSL-2 facility. All study protocols were reviewed and approved by the Zoetis Institutional Care and Use Committee before study start.

Experimental Design and Randomization

Two separate, randomized, controlled studies with 40 calves per study were conducted to evaluate the efficacy of the *att*BVDV-1 and *att*BVDV-2 fractions in a modified-live virus-toxoid vaccine administered as a single SC dose, for protection of young calves ~ 60 days of age, against virulent BVDV-1 or BVDV-2 challenge. For each study, there were 2 treatment groups (vaccinated or placebo group), each containing 20 animals. The study design was a generalized randomized block design with 1-way treatment structure. Blocks were based on room assignment during the challenge phase. Animal was the experimental unit during both vaccination and challenge phases of the study. During the vaccination phase of the study, animals were housed in individual pens, and animals were randomly allocated to treatments per a completely randomized design. During the challenge phase of the study, animals were allocated to challenge rooms per a generalized block design with 1-way treatment structure. Animal randomization plans to treatments and challenge rooms were generated by a Zoetis biometrics representative. The random treatment allocation plan was created using a SAS statistical program[c] that utilized a random number generator function. Study inclusion criteria required that all calves were clinically healthy, not persistently infected with BVDV, and seronegative for antibodies against BVDV-1 and BVDV-2 (serum VN antibody titer < 1:2 on day of vaccination and negative IHC from ear notch submitted to Iowa State University Diagnostic Laboratory prior enrollment).

Vaccination and Challenge

In each study, calves in the vaccine treatment group received a combination ML BHV-1, BVDV, PI_3V, BRSV vaccine + *Mannheimia haemolytica* toxoid.[d] Animals in the control group received a placebo formulated in the same way as the vaccine, but without the *att*BVDV-1a and *att*BVDV-2 fraction. The respective vaccine fractions were titrated for an input level below the established minimum immunizing dose (MID) of the commercial product.[d] A single, 2-mL (vaccine or placebo) dose was administered SC in the neck region to animals at ~60 d of age (Table 1). On d 35 (Study 1) and d 38 (Study 2) post-vaccination, individual calves were challenged IN with either virulent non-cytopathic BVDV-1b, strain NY-1 (6.93 log_{10} $TCID_{50}$/ mL dose; Study 1) or virulent non-cytopathic BVDV-2 strain #24515 (5.4 log_{10} $TCID_{50}$/mL dose; Study 2). A compressed gas atomizer was used to administer either a 4-mL dose (2 mL per nostril; Study 1) or a 5-mL dose (2.5 mL per nostril; Study 2) of the challenge material to each calf. At the end of each study, all animals were humanely euthanized in compliance with American Veterinary Medical Association Guidelines for Humane Euthanasia, and disposed by secure burial.

Clinical Assessment

In Studies 1 and 2 following challenge with virulent BVDV-1 or BVDV-2, respectively, calves were monitored for 14 consecutive days for presence of pyrexia (≥103.5°F; 39.7°C) and clinical signs related to BVDV disease (depression, dyspnea, nasal discharge, diarrhea, hypersalivation). Trained personnel performing clinical observations were blinded to treatment groups. Scoring of clinical signs related to respiratory disease was performed as described in Table 2, and used to determine severity of disease post-challenge.

Leukopenia was defined as a ≥40% reduction in total white blood cell (WBC) count compared to the baseline WBC level. This clinical definition of leukopenia was established internally and was reviewed and accepted by the USDA Center of Veterinary Biologics as an outcome variable determining the success of a BVDV challenge and vaccine efficacy. Baseline WBC values were determined as an average of individual calf's WBC for 3 consecutive days prior to challenge.

Virus Neutralization (VN) Assay

To evaluate serum neutralizing antibody levels post-vaccination and post-challenge, a whole-blood sample was collected from a jugular vein of each calf. Animals were bled prior to vaccination (d -1) and study d 21, 27, prior to viral challenge, and at the end of study for serology analysis. Serum samples were harvested and stored at 39°F (4°C) until tested for presence of neutralizing antibodies to BVDV-1 and BVDV-2 in a virus neutralization (VN) test as previously described.[24]

Briefly, serum samples were heat-inactivated at 133°F (56°C) for 30 minutes, vortex mixed, and serially diluted (1:2) in assay diluent (Dulbecco's Modified Eagle Medium[e] [DMEM] supplemented with L-glutamine [2mM], gentamicin [20 mg/L], and donor horse serum [5%]). Following titration, an equal volume of BVDV-1 or BVDV-2 was added to yield 50 to 300 $TCID_{50}$/well, and plates were incubated for 60 min at room temperature. A total of 200 µL of serum-virus mixture at each dilution was added in a quadruplicate to individual wells in a 96-well tissue culture plate containing bovine turbinate (BT) cells at a confluency of >95%. Following incubation for 6 to 8 d at 98.6°F (37°C) in 5% CO_2, wells were examined for the presence of BVDV cytopathic effect (CPE). The neutralizing antibody titer for each sample was calculated using the Spearman-Karber method.

Qualitative Virus Isolation from Peripheral Blood Mononuclear Cells (PBMCs)

For purpose of detecting viremia, whole-blood samples were collected in tubes containing EDTA on day of challenge and daily for 14 d post-challenge. Virus isolation was performed from PBMC samples, and presence of BVDV-1 and BVDV-2 viruses in tissue culture was confirmed by fluorescent antibody staining. Two mL of whole blood were added to 2.0 mL of phosphate buffered saline (PBS) in an ACCUSPIN™[f] 12 mL tube containing density gradient separation medium Histopaque®-1077 below the frit. Tubes were centrifuged at 800

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 2. Clinical scoring system for severity of BVDV clinical disease.

Clinical Evaluation	Clinical Score
Depression	**0 = Absent 1 = Mild:** Animal tends to stand with head lower than roommates. Has a dull appearance in one or both eyes; one or both ears may droop lower than ears of pen mates. **2 = Moderate:** Animal is lethargic with movements and responses to stimuli that are slow, hesitant or unsteady. Animal has a reduced interest in surroundings, feed and may stand off from pen mates or from feed. **3 = Severe:** Animal maybe recumbent and is markedly slower in rising and rises (maybe unsteadily) with increased effort. Animal is anorexic.
Respiratory Effort	**0 = Absent 1 = Mild:** Increased abnormal respiration depicted by slight increased respiratory rate **2 = Moderate:** Respiratory effort may be deep, and primarily abdominal or markedly shallow and rapid (Tachypnea) **3 = Severe:** Breathing may be audible as raspy or with an expiratory "grunt" during exhalation. (Dyspnea)
Nasal Discharge	**0 = Absent 1 = Mild:** Small to notable amount of serous discharge accumulated in or running out of the nostrils. **2 = Moderate/Severe:** Notable amount (approximately ≥ 5 mL) of persistent serous or mucopurulent discharge accumulated in or running out of the nostrils.
Diarrhea	**0 = Absent 1 = Mild:** Stool is loose in consistency, mushy or thick liquid consistency. **2 = Moderate/Severe:** Stool is watery with no solid pieces and little to no consistency. Animal becomes dehydrated.
Hypersalivation	**0 = Absent 1 = Mild:** Small to notable amount of salivation. **2 = Moderate/Severe:** Animal has excessive saliva or foam running out of or around its mouth.

x g for 15 min at room temperature. Plasma layers were then discarded, and the leukocyte band (buffy coat) was added to a 15 mL centrifuge tube containing 10 mL of PBS and centrifuged for 10 min at 250 x g. The supernatant was discarded, and the buffy coat pellet was resuspended in 1 mL of viral transport media[e] and antibiotic-antimycotic (1X). Samples were stored frozen at ≤-274°F (≤-70°C) until tested. Confluent (>95%) monolayers of bovine turbinate (BT) cells in 96-well tissue culture plates were used for BVDV-1 or BVDV-2 virus isolation from the thawed, resuspended buffy coat samples. A total of 60 µL of each sample was added to 1 column of a 96-well plate. Plates were incubated for 6 to 8 d at 98.6°F (37°C) in 5% CO_2. Following incubation, plates were frozen at ≤-274°F (≤-70°C) until subsequent subculture. Frozen plates were thawed and subcultured by stamping 60 µL onto a fresh 96-well plate with confluent (>95%) BT cells. Plates were incubated for 6 to 8 days at 98.6°F (37°C) in 5% CO_2. After incubation, plates were fixed and stained using an indirect fluorescent antibody (FA) procedure with mAbs 12g4 (BVDV-1) and Bz-53 (BVDV-2). Plates were read for the presence/absence (qualitative read) of BVDV. The results were reported as Yes or No for virus presence, but no virus quantitation was performed.

Leukocyte Counts

Whole-blood samples were collected in tubes containing EDTA daily beginning 3 d before challenge and through 2 weeks post-challenge for leukocyte counts. Blood samples were analyzed within 24 h of collection using the ADVIA 120 hematology analyzer.

Statistical Analysis

A designated VMRD-biometrician was responsible for data summaries and analyses of data entered into the centralized data management system.[c] Prevalence of clinical disease in each of the 2 studies was compared between vaccinated and control groups with the 2-tailed Fisher exact test. Duration of respiratory clinical signs was calculated as "the date the signs were last noticed, minus the date the signs were first noticed, plus 1". Duration of viremia was determined for each animal and was calculated as "last time-point present minus first time-point present plus 1". Duration of viremia was set to zero for animals that had no time-points with positive viremia. Duration of viremia was calculated as "last scheduled time-point of virus isolation collection minus first time-point present plus 1" for animals that were removed from the study prior to the last scheduled VI collection timepoint. Durations were subsequently compared between treatment and control groups in each experiment with a general linear mixed model, with treatment as a fixed effect and challenge room and the residuals as random effects. Data on viremia, challenge-phase rectal temperatures, total WBC, and challenge-phase antibody titers were each compared between groups within each study with a general linear mixed model with repeated measures, with treatment, assessment point, and the interaction between these 2 variables as fixed effects and challenge room, individual calf within challenge room, and residuals as random effects. Least squares means (LSM) values of $P \leq 0.05$ were considered significant for all analyses.

Results

Vaccine Safety and Animal Removal

A total of 80 calves (40 per each study) were vaccinated at ~60 d of age with either the 5-way MLV/toxoid vaccine (20 per study) or placebo without *att*BVDV-1 and *att*BVDV-2. No animal in either study showed signs of an adverse reaction (including but not limited to site reactions, fever, anaphylaxis, and tremor) post-administration of corresponding treatments (vaccine or placebo). In Study 1, 1 animal from the vaccinated group was removed from the study on d 35

© Copyright American Association of Bovine Practitioners; open access distribution.

(prior to challenge) due to severe laminitis, which is often seen when calves are transitioned from straw bedding to concrete flooring.[3] In Study 2, a total of 6 animals were removed from the study before completion due to severity of disease (clinical scores >3) associated with BVDV-2 challenge. In the placebo group, 1 animal was found dead on d 49 (11 d post-challenge) and 3 animals were humanely euthanized on d 49, 49, and 51, respectively. In the vaccinated group 2 animals were humanely euthanized on d 49 and 51, respectively (d 11 and 13 post-challenge).

Post-challenge Leukopenia

Study 1 *att*BVDV-1a efficacy

Mean WBC counts in the placebo group were significantly lower starting 3 d post-challenge (d 38 to 42 and 44 to 49; Figure 1A) compared to the vaccinated group. Furthermore, 15 of 20 control calves (75%) developed leukopenia, compared to only 5 of 19 (26.3%) vaccinated calves (p=0.0063) after the BVDV 1 challenge. On d 6 and 10 to 12 d post-challenge (study d 41 and 45 to 47) there were significantly fewer leukopenic animals in the vaccinate group compared to placebo group (Figure 1C, Table 4A). Numerical differences in reduced number of leukopenic animals were seen in the vaccinated group compared to calves in the placebo group on other study days, but without statistical significance (Table 4A). Another significant observation was reduction in mean duration of leukopenia post-challenge in the vaccinated group compared to placebo, with duration of leukopenia of 1.3 d in vaccinates compared to 4.7 d in the placebo group (p=0.009).

Study 2 *att*BVDV-2 efficacy

Similar to Study 1, at 3 d post-challenge (d 41) mean WBC counts were significantly lower in the placebo group compared to vaccinated animals, and remained low until the end of the study (Figure 1B). Overall, there were significantly fewer (p=0.0001) leukopenic animals in the vaccinated group post-challenge with virulent BVDV-2, as 100% of the control calves developed leukopenia compared to only 25% of the vaccinated calves. Furthermore, from d 3 post-challenge (study d 41) until the end of the study there was a significant daily reduction in the number of leukopenic animals in the vaccinated group compared to the placebo-treated animals (Figure 1D, Table 4B). The average duration of leukopenia post-challenge in the vaccinated group was significantly shorter (2.6 d) compared to the placebo group (11.3 d; p<0.0001).

Post-challenge Viremia

Vaccine efficacy in both studies was assessed by comparing the presence of live virus in PBMC and the duration of viremia between the placebo and vaccinated groups following challenge.

Study 1. *(BVD-1b viremia)* Following challenge, all 20 (100%) control calves were viremic compared to 11 of 19 (57.9%) vaccinated calves (p=0.0012). From d 3 to 11 post-challenge there was a significant reduction in the daily number of viremic calves in vaccinated calves compared to placebo-treated calves (Figure 2A, Table 4A). Duration of viremia in vaccinates was significantly shorter (least squares mean of 1.7 d) compared to the viremia duration in placebo calves (least squares mean of 6.9 d; p<0.0001).

Study 2. (*BVDV-2 viremia)* Similar to Study 1, after challenge with virulent BVDV-2 virus, all 20 (100%) control calves were viremic compared to only 5 of 20 (25%) vaccinated calves (p=0.0001). There was a significant reduction in the number of viremic animals from d 3 to d 14 post-challenge (Study d 41 to 52) in the vaccinated group compared to placebo controls (Figure 2B, Table 4B). Furthermore, mean duration of viremia was significantly shorter (p<0.0001) in vaccinated calves (1.4 d of viremia) compared to the placebo group (11.7 d of viremia).

Serology

All calves were seronegative (VN titer <1:2) to BVDV-1 and BVDV-2 antigen prior the start of Study 1 and Study 2, and the placebo-treated animals remained seronegative prior to challenge.

Study 1. At 21 d post-vaccination, 17 of 20 vaccinates had seroconverted (titer ≥2) with a LSM BVDV-1 titer of 2±0.7, which was not significantly different than the placebo group (p=0.17). The virus neutralizing (VN) titers of vaccinated groups increased further, and on d 34 (day prior challenge) the LSM BVDV-1 titer was 27±7, which was significantly higher (p<0.0001) than LSM BVDV-1 titers of calves in the placebo group (LSM titer of 1±0.2; Figure 3A). Following viral challenge, all calves in the vaccine group demonstrated evidence of anamnestic response and robust increase in antibody titers. The LSM value of BVDV-1 VN antibody titers for vaccine-treated calves had increased from 27±7 to 2658 ±670.5, which was significantly higher (p<0.0001) compared to VN LSM BVDV-1 titer of 223±24.6 observed in control calves at the end of the study (d 49).

Study 2. Vaccination with MLV vaccine containing *att*BVDV-2 fraction induced seroconversion in 10 of 20 calves on d 21 post-vaccination (LSM BVDV-2 titer 4±1.6) and in 18 of 20 calves by pre-challenge d 37 (LSM BVDV-2 titer 315±100.7; Figure 3B), which was significantly higher compared to the placebo group (p<0.0001). On d 52 (14 d post challenge), BVDV-2 neutralizing antibody titers of the vaccinated calves were significantly higher (LSM BVDV-2 titer 4694±1580.0; P<0.0001) compared to the control group (LSM antibody titer of 337±48.5).

Clinical Assessment

Study 1 (*att*BVDV-1 efficacy)

Following challenge with the virulent BVDV-1b, mild clinical signs related to BVDV-1b infection were observed, which was expected knowing that the challenge virus does not induce robust clinical disease. Diarrhea was observed

© Copyright American Association of Bovine Practitioners; open access distribution.

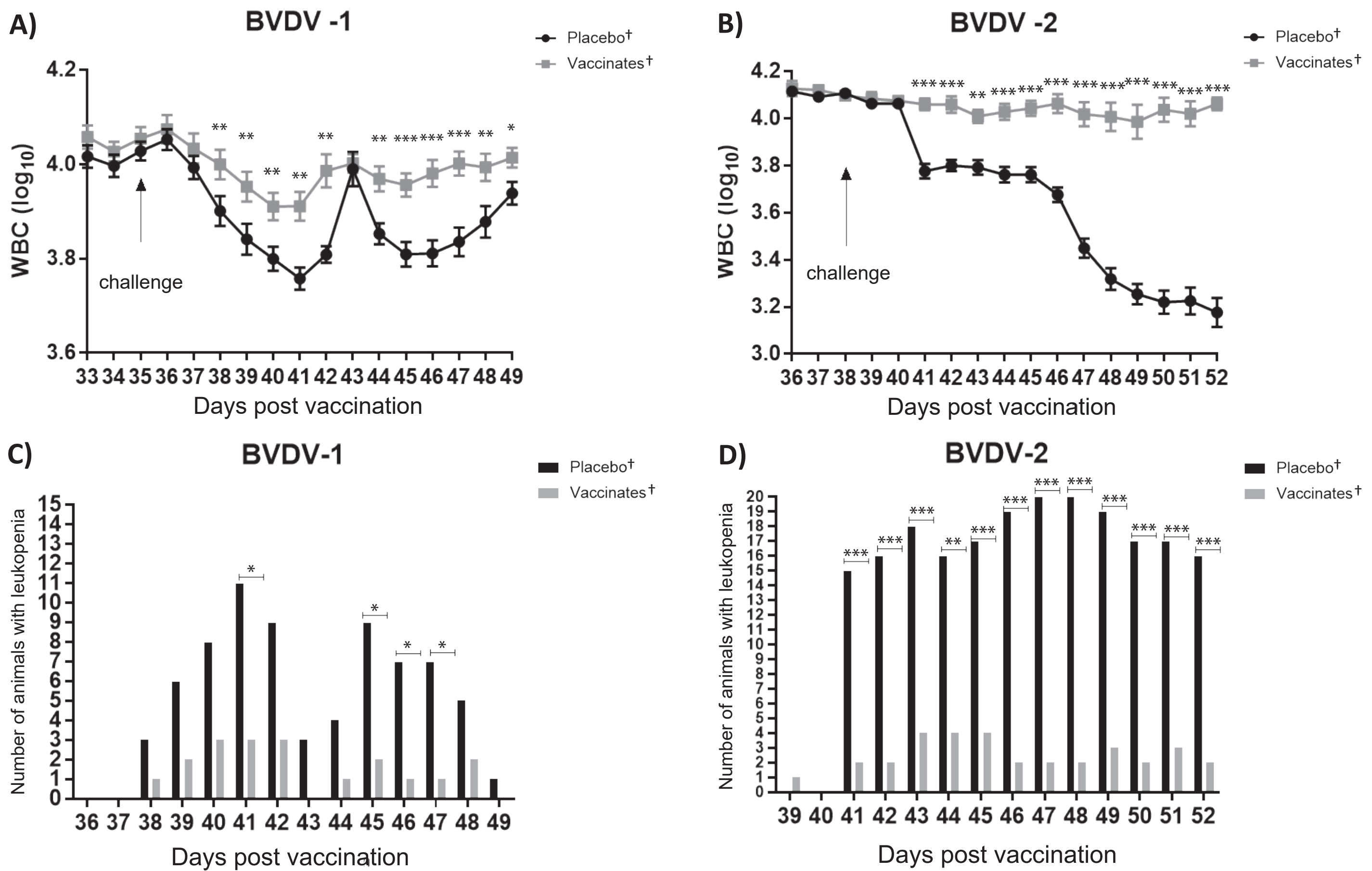

Figure 1. WBC counts in calves following vaccination with a combination multivalent MLV-toxoid vaccine + *Mannheimia haemolytica* toxoid and challenged with virulent BVDV-1b (A) or BVDV-2 (B). Number of animals with leukopenia per day of study following challenge with BVDV-1b (C) and BVDV-2 (D). WBC counts expressed as Log_{10} value. Leukopenia defined as >40% decrease in leukocytes post-challenge compared to baseline (mean WBC for 3 consecutive days pre-challenge). Data points significantly different if p<0.0001(***); p<0.001 (**) and p<0.05 (*). Error bars shown as error of the mean (SEM).

†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

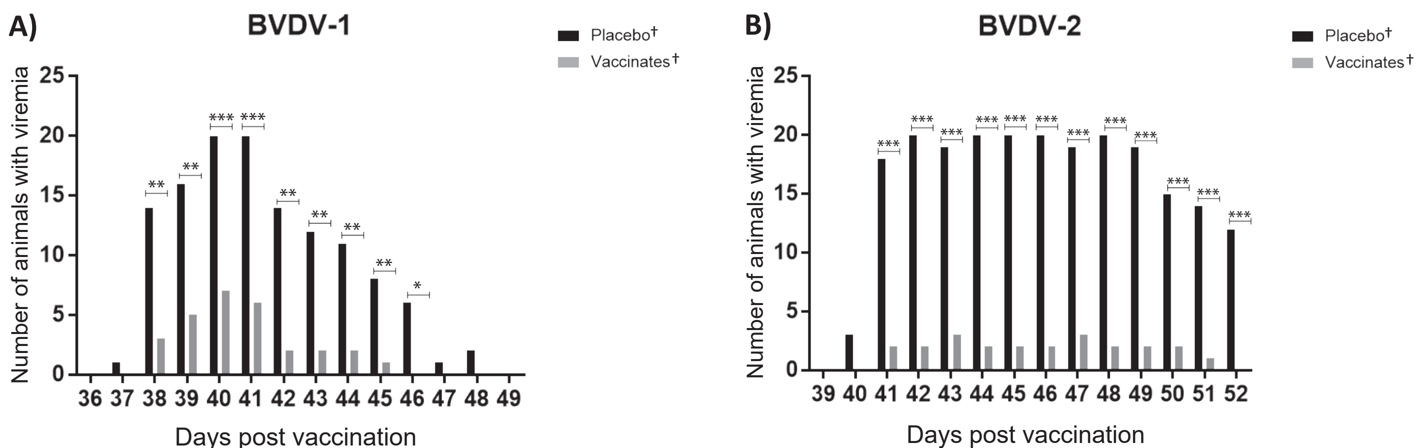

Figure 2. Number of animals positive for live virus (viremia) in PBMC following challenge with BVDV-1b; Study 1 (A) and BVDV-2; Study 2 (B). Data points significantly different if p<0.0001(***); p<0.001 (**) and p<0.05 (*).

†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

© Copyright American Association of Bovine Practitioners; open access distribution.

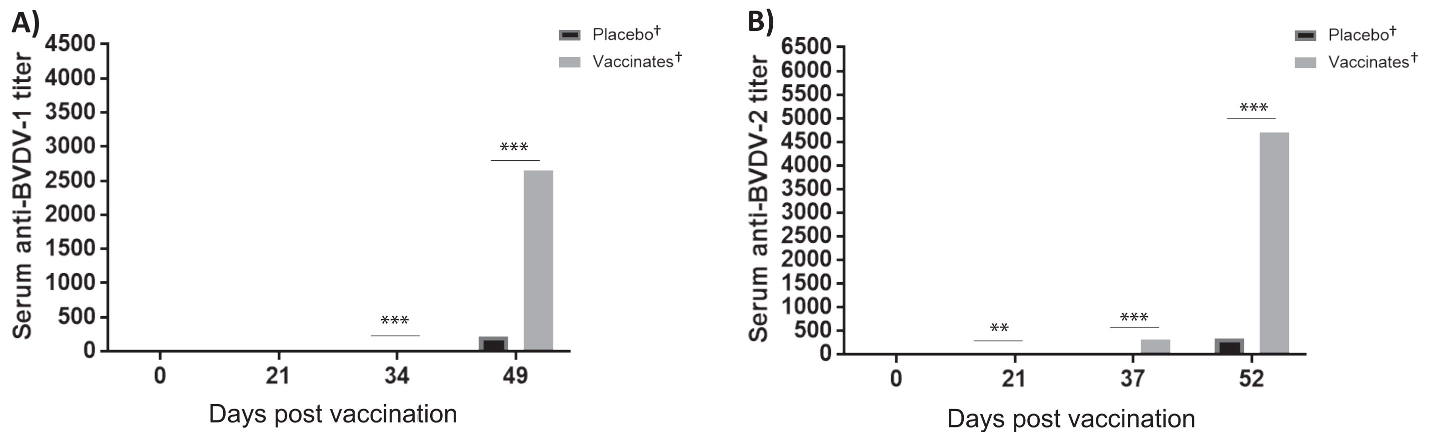

Figure 3. LSM virus neutralizing serum antibody titers against (A) BVDV-1 (Study 1) and (B) BVDV-2 (Study 2), at pre-vaccination (day 0), post-vaccination (day 21, 34 [BVDV-1], day 37 [BVDV-2]) and post-challenge (day 49 [BVDV-1], day 52 [BVDV-2]). Data points significantly different if p<0.0001 (***); p<0.001 (**) and p<0.05 (*). Error bars shown as error of the mean (SEM).
†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

in 10% of placebo calves and 5% of vaccinates during post-challenge observation (not significant). A small portion of placebo-treated calves (30%) showed clinical signs of depression post-challenge compared to 10% of vaccinates (not significant). Hypersalivation and respiratory effort were not observed in either of the 2 treatment groups. While abnormal nasal discharge was not present in vaccinates, it was observed in 20% of placebo calves. There was no significant reduction in number of calves with diarrhea, depression, hypersalivation, nasal discharge, and respiratory effort on 1 or more time points during the post-challenge observations in vaccinates compared to placebo calves (Table 3A); however, there were numerical differences with a trend of lower numbers of calves in the vaccinated group displaying clinical signs compared to placebo-treated calves (Table 3A).

Mean rectal temperature in vaccinates was significantly lower on post-challenge days 3, 7, 8, and 10 (study d 38, 42, 43, and 45) compared to the placebo group (Figure 4A). A total of 55% (11/20) of placebo calves developed pyrexia (≥103.5.0°F; ≥39.7°C) for at least 2 days, compared to 26% of (5 of 19) vaccinated calves (p=0.0832). In addition, vaccinated calves had shorter mean duration of pyrexia compared to placebo calves (1.3 d vs 2.5 d; p=0.052).

Study 2 (attBVDV-2 efficacy)

The *att*BVDV-2 vaccine fraction induced significant protection against characteristic BVDV disease. Diarrhea was observed in 95% of placebo calves compared to 15% of vaccinates (p=0.0001) during post-challenge observation. All placebo-treated animals showed clinical signs of depression post-challenge compared to 20% of vaccinates (p=0.0001). Hypersalivation was seen in 55% of placebo-treated animals and 10% of vaccinates (p=0.0057); abnormal nasal discharge was observed in 60% of placebo calves at least once following challenge, compared to 5% of vaccinates (p=0.0004). Increased respiratory effort was observed in 75% of calves in the placebo group vs 45% in vaccinates, which was not statistically significant (p=0.10).

In addition, there was significant reduction in the number of animals with diarrhea, depression, hypersalivation, nasal discharge, and respiratory effort on 1 or more observation time points post-challenge in the vaccinated group compared to placebo treated animals (Table 3B).

From 3 d post-challenge (d 41) until end of the study (d 52) there was a reduction (P ≤ 0.04) in mean rectal temperatures in the vaccinated group compared to the placebo group (Figure 4B). Following challenge with virulent BVDV-2, mean duration of pyrexia in the placebo group was 10.1 d compared to 1.7 d in the vaccinated group (p<0.0001). Furthermore, daily number of animals with pyrexia post-challenge was significantly higher in the placebo group compared to the vaccinates, indicating protective efficacy induced by the BVDV-2 vaccine fraction (Table 3B) on d 41 and 43 to 52. In addition, 20 of 20 (100%) placebo-treated, control calves developed pyrexia (≥103.5.0°F; ≥39.7°F) for at least 2 days, whereas only 3 of 20 (15%) vaccinated calves showed clinically relevant pyrexia (p=0.0001).

Discussion

Since the first appearance as clinical disease in the 1940s, BVDV infection in cattle has presented numerous challenges for veterinarians and researchers worldwide.[15] Strategies to control BVDV-related disease are complicated by genetic variation between viral strains, viral tropism for epithelial, hematopoietic and immune cells, the establish-

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 3. Analysis of presence of clinical signs at each time point; data presented as percentage of animals with clinical signs per each day post-challenge with A) BVDV-1b (Study 1) and B) BVDV-2 (Study 2). ****significant difference (P <= 0.05)***

A)

Days post vaccination		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Days post-challenge		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhea	**Placebo†**	0	0	0	0	0	0	0	0	0	**5**	**5**	**5**	0	**5**	**10**
	Vaccinates†	0	0	0	0	0	0	0	0	0	0	0	0	**5.3**	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Depression	**Placebo**	0	0	0	0	0	0	0	**5**	**20**	**10**	0	0	**5**	**5**	**5**
	Vaccinates	0	0	0	0	0	0	0	0	0	**10.5**	**5.3**	0	0	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Hypersalivation	**Placebo**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Vaccinates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Nasal discharge	**Placebo**	0	0	0	0	**0**	0	0	**10**	**10**	**15**	**5**	**5**	0	0	0
	Vaccinates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Respiratory effort	**Placebo**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Vaccinates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Fever	**Placebo**	0	0	**5**	**15**	**10**	0	0	**75**	**45**	**5**	0	0	0	0	0
	Vaccinates	**5**	0	0	**10.5**	0	0	**10.5**	**42.1**	**15.8**	**15.8**	**5.3**	0	0	0	0
	P value	*ns*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

B)

Days post vaccination		**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**
Days post-challenge		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Diarrhea	**Placebo†**	**5**	0	0	0	0	0	0	0	0	**5**	**15**	**55**	**52.9**	**70.6**	**81.3**
	Vaccinates†	0	0	0	0	0	0	0	0	0	0	0	**10**	0	**5.3**	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.006***	***0.0003***	***0.0001***	***0.0001***
Depression	**Placebo**	0	0	0	0	0	0	0	0	0	0	**65**	**95**	**94.1**	**88.2**	**93.8**
	Vaccinates	0	0	0	0	0	0	0	0	0	0	**10**	**15**	**10.5**	**10.5**	**0**
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.0008***	***0.0001***	***0.0001***	***0.0001***	***0.0001***
Hypersalivation	**Placebo**	0	0	0	0	0	0	0	0	0	0	**5**	**30**	**29.4**	**29.4**	**18.8**
	Vaccinates	0	0	0	0	0	0	0	0	0	0	**5**	0	0	**5.3**	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.02***	***0.02***	*ns*	*ns*
Nasal discharge	**Placebo**	0	0	10	0	**0**	0	0	0	0	**5**	**10**	**20**	**47.1**	**17.6**	**25**
	Vaccinates	0	0	0	0	0	0	0	0	0	0	0	**5**	0	0	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.0008***	*ns*	***0.04***
Respiratory effort	**Placebo**	0	5	0	0	0	0	0	0	0	0	0	**50**	**58.8**	**52.9**	**37.5**
	Vaccinates	10	5	0	0	0	0	0	0	0	0	**5**	**20**	**21.1**	**21.1**	**5.6**
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.04***	*ns*	***0.03***
Fever	**Placebo**	0	0	0	**40**	**5**	**35**	**60**	**85**	**100**	**100**	**90**	**94.7**	**88.2**	**76.5**	**68.8**
	Vaccinates	0	0	0	**5**	0	**5**	**10**	**15**	**15**	**10**	**10**	**25**	**10.5**	**5.3**	0
	P value	*ns*	*ns*	*ns*	***0.01***	*ns*	***0.04***	***0.002***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***

†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 4. Analysis of leukopenia and viremia; data presented as percentage of animals with clinical signs per each day post-challenge with A) BVDV-1b (Study 1) and B) BVDV-2 (Study 2). **significant difference (P <= 0.05)*

A)

Days post vaccination		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Days post-challenge		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Leukopenia	**Placebo†**	0	0	0	15	30	40	55	45	15	20	45	35	35	25	5
	Vaccinates†	0	0	0	5.3	10.5	15.8	15.8	15.8	0	5.3	10.5	5.3	5.3	10.5	0
	P value	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***0.02***	*ns*	*ns*	*ns*	***0.03***	***0.04***	***0.04***	*ns*	*ns*
Viremia	**Placebo**	0	0	5	70	80	100	100	70	60	55	40	30	5	10	0
	Vaccinates	0	0	0	15.8	26.3	36.8	31.6	10.5	10.5	10.5	5.3	0	0	0	0
	P value	*ns*	*ns*	*ns*	***0.0011***	***0.0012***	***0.0001***	***0.0001***	***0.0002***	***0.002***	***0.006***	***0.02***	***0.02***	*ns*	*ns*	*ns*

B)

Days post vaccination		38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Days post-challenge		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Leukopenia	**Placebo†**	0	0	0	75	80	90	80	85	95	100	100	100	100	100	100
	Vaccinates†	0	5	0	10	10	20	20	20	10	10	10	15	10.5	15.8	11.1
	P value	*ns*	*ns*	*ns*	***0.0001***	***0.0001***	***0.0001***	***0.0004***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***
Viremia	**Placebo**	0	0	15	90	100	95	100	100	100	95	100	100	88.2	82.4	75
	Vaccinates	0	0	0	10	10	15	10	10	10	15	10	10	10.5	5.3	0
	P value	*ns*	*ns*	*ns*	***0.0001***	***0.0001***	***0.0001***	***0.0004***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***	***0.0001***

†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

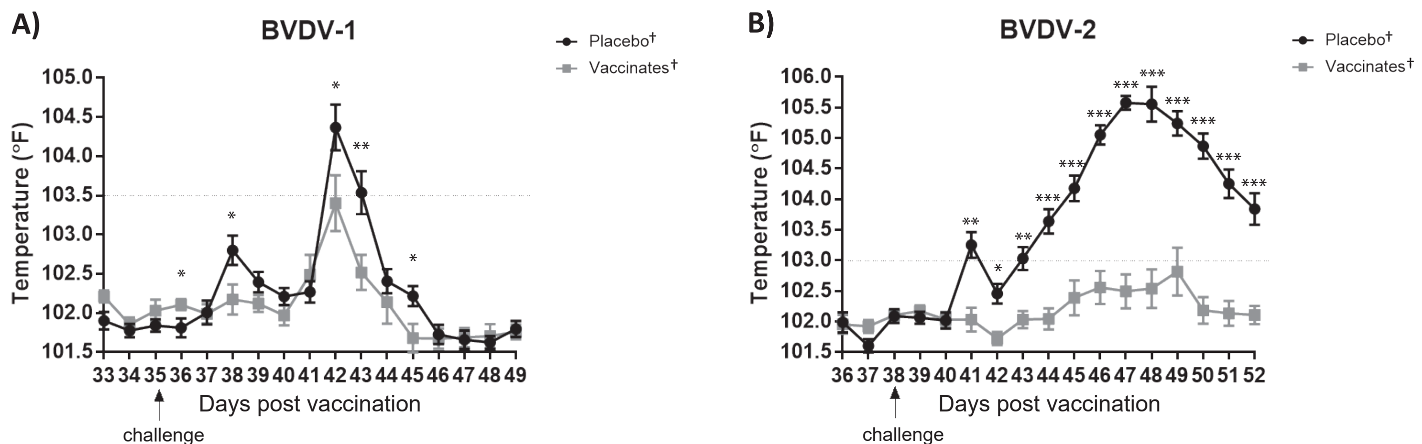

Figure 4. Least squares means (LSM) of rectal temperatures in calves following vaccination with a combination multivalent 5-way MLV vaccine + *Mannheimia haemolytica* toxoid and challenged with virulent BVDV-1b (A) or BVDV-2 (B) temperatures unit expressed in Fahrenheit. Data points significantly different if p<0.0001(***); p<0.001 (**) and p<0.05 (*). Error bars shown as error of the mean (SEM).
†Vaccinates administered a modified-live BHV-1, BVDV (types 1 and 2), PI3V, BRSV vaccine + *Mannheimia haemolytica* toxoid (Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ). In the placebo vaccine, test antigen fractions *att*BVDV-1a and *att*BVDV-2 were removed.

ment of persistent infections, and various clinical forms of disease. BVDV infection can occur by either the aerosol route or fecal–oral transmission, and the primary targets of infection are epithelial cells in the oral cavity, respiratory tract, reproductive tract or gastrointestinal tract. Once this mucosal barrier is breached, virus spreads to mucosal associated lymphoid tissue (i.e., Peyer's patches, draining lymph nodes).[22] Viremia is also a common observation resulting in

© Copyright American Association of Bovine Practitioners; open access distribution.

a systemic infection targeting a wide variety of tissues. Of particular importance is the infection of hematopoietic and lymphoid tissues that results in leukopenia and immune suppression. Overall, clinical representation of BVDV infection is not completely clear due to involvement of multiple organ systems and could be varying from subclinical infections or mild clinical disease to severe fatal syndromes. Besides BVDV being an important contributor to the BRD complex, BVDV causes significant economic losses as a reproductive disease, such as abortions resulting in loss of pregnancy, but the virus can be transmitted to the fetus if the dam is infected from 45 to 125 days of gestation. Fetal transmission of non-cytopathic BVDV leads to the birth of persistently infected (PI) calves which are the consistent source of BVDV in herds.

Vaccines have been used at the forefront of BVDV disease control strategy for more than 50 years,[4] and vaccination protocols addressing the need to target specific cattle populations and monitor disease prevalence have been established.[4,16] Currently, killed and MLV vaccines have been developed that specifically prevent BVDV disease related to BRD and fetal transmission.

BVDV has been divided into 2 major genotypes, BVDV-1 and BVDV-2, but each genotype is further divided into subtypes according to their genetic and antigenic differences.[26,33]

This genetic variability poses several challenges for vaccine development, and consequently its safety and efficacy. Viral isolates selected for vaccine development are chosen for their ability to provide cross-protection against the diverse species and strains circulating within herds. As a result, most of the current vaccine formulations include both a BVDV-1 and BVDV-2 isolate, and vaccine efficacy studies frequently include serology to evaluate neutralization of heterologous virus species and animal challenge studies with a heterologous strain.[37,42] In addition, researchers and field veterinarians have raised concerns about the efficacy of vaccines containing BVDV-1a antigen against BVDV-1b infections, and underscoring the point that efficacious vaccines, particularly controlling BVDV-1b infections, may be required.[12]

In the current study we explored efficacy of a MLV 5-way virus-toxoid vaccine to confer protection against experimentally induced heterotypic BVDV-1b and BVDV-2 infection in young calves at ~ 60 d of age following a single SC vaccination. BVDV-1b subtype was more frequently associated with BRD disease, and the predominant isolate at necropsies in calves with severe bronchopneumonia.[12,13] Therefore, even though our vaccine formulation was based on BVDV-1a antigen, the decision was made to challenge calves with a heterosubtypic strain of BVDV-1b genotype. In general, on its own, wild-type BVDV-1b viruses under experimental conditions induce mild clinical representation (pyrexia, diarrhea, depression); however, they are able to consistently induce leukopenia and viremia as hallmarks of BVDV infection.[13] As seen from the data presented here, single SC administration of 5-way virus-toxoid vaccine was sufficient in inducing a significant serologic immune response to vaccine antigen prior to the challenge. Furthermore, post-challenge antibody responses indicated significant anamnestic serologic response despite heterosubtypic challenge with the BVDV-1b strain. In addition, post-challenge antibody responses correlated with significant reduction of viremia, and consequently leukopenia, in vaccinated animals compared to placebo-vaccinated controls, which were considered as the primary variables defining efficacy.

Since the early 1990s, a syndrome of severe acute condition with high morbidity and mortality in susceptible animals linked to BVDV-2 has been described. Primary infections with highly virulent BVDV-2 strains were linked to this syndrome characterized by peracute to acute course and signs of fever, diarrhea, pneumonia, and sudden death. The pathogenesis of BVDV-2 is most often associated with increased strain virulence.[9,23] Similar to natural infection, reproduction of severe acute BVDV-2 related disease in experimental conditions by intranasal administration is characterized by transient pyrexia at 48 to 72h post-infection and unremitting pyrexia starting 6 to 8 days post-challenge. Pyrexia is further accompanied by watery diarrhea and depression as marked clinical observations following BVDV-2 challenge.[7] Consistent with these observations, in the current study severe acute BVDV disease was observed in the placebo group after challenge with virulent BVDV-2, characterized by prolonged pyrexia, depression, watery diarrhea, excessive viremia, and leukopenia. In contrast, the vaccinated group showed significant, marked reduction in incidence and duration of pyrexia, leukopenia, and viremia following challenge with the BVDV-2 strain. In addition, vaccinated animals showed significant reduction in other clinical signs such as depression, nasal discharge and diarrhea, indicating clear protective efficacy. Protection from BVDV-2 infection is mediated in part by the detectable presence of mucosal and serum VN antibodies after exposure to live virus as well as a reduction in incidence and duration of leukopenia and viremia. In Study 2, a single SC vaccination induced seroconversion in vaccine-treated calves by the time of challenge administration (d 37) with significant ($p<0.0001$) VN antibody titers compared to calves in the placebo group. The re-exposure to homotypic BVDV-2 strain post-challenge resulted in accelerated anamnestic serum antibody responses reaching significantly higher neutralizing antibody titers. Furthermore, the role of serologic antibody responses in protection from BVDV-2 challenge was demonstrated in the current study. Two animals which had not responded to the vaccine (<1:2 serum VN antibodies) developed severe clinical signs and were euthanized. The vaccine was able to induce protective immunity against BVDV-2 challenge in vaccinated calves, resulting in a significant reduction in the number of viremic and leukopenic animals and those showing diarrhea and respiratory clinical signs, compared to the placebo group.

Altogether, the studies demonstrated that in response to BVDV-1b and BVDV-2 challenge viruses, vaccinated calves were protected from developing viremia, leukopenia, and clinical signs of disease. While all controls developed BVDV-

© Copyright American Association of Bovine Practitioners; open access distribution.

1b and BVDV 2 viremia, significantly fewer vaccinates (57.9% and 25%) developed viremia and for a shorter duration. Control of viremia (duration and frequency) is important for BVDV control as it would reduce shedding into the environment and also prevent the ill health effects induced by BVDV. In respect to duration, vaccinated calves in Study 1 (BVDV-1) and Study 2 (BVDV-2) shed virus for 1.7 and 1.4 d, compared to controls which shed infective virus for 6.9 and 11.7 d, respectively. Similarly, the effect of BVDV infection on total WBC counts was limited, with frequency and duration of leukopenia affecting significantly fewer calves in the vaccinate group and for a shorter time in both Study 1 and 2, compared to controls (duration of leukopenia Study 1: vaccinates 1.3 d vs controls 4.9 d; Study 2: vaccinates 2.6 d vs controls 11.3 d).

Overall, these observations strongly suggest that vaccine containing BVDV-1a and BVDV-2 antigens are protective against heterotypic BVDV-1b and BVDV-2 challenge in young calves ~60 d of age.

Conclusions

Data reported here confirmed that a single SC dose of the multivalent, MLV/toxoid vaccine modelled on a commercial vaccine was safe as no adverse effects associated with vaccine administration to 60-day-old calves were noted. Furthermore, a single dose of this vaccine induced clinically relevant disease-sparing protective immunity against BVDV-1b and BVDV-2 respiratory challenge in naïve 60 d-old calves. This age is linked to branding activities and is convenient timing for vaccinating calves, building immunity against key pathogens ahead of their next phase of life prior to weaning. The earliest opportunity to vaccinate beef and dairy calves is at birth; however, this time point is often challenged both logistically and immunologically. Calves rely on the maternal colostral antibodies for protection against multiple pathogens, and with time they may lose this passive protection and become susceptible to multiple diseases. Failure of passive transfer of antibodies is a well-known phenomenon, and the 60 d of age period is a vulnerable time, thus highlighting the importance of ensuring continuity of calf protection against key pathogens. Branding is a longstanding management practice that includes a series of vaccinations and management practices at various age stages in order to better prepare beef calves for their transition to subsequent production sectors within the industry. In this report we demonstrated that modified-live BHV-1, BVDV, PI3, BRSV vaccine + *Mannheimia haemolytica* toxoid is safe and efficacious in young calves 60 d of age following single SC against BVDV-1 and BVDV-2 challenge viruses.

Additional research is required to better characterize the duration of immunity, cell-mediated component of the immune responses, vaccine efficacy in the presence of maternal antibodies, and the potential to extend or improve the response by re-vaccination with additional mucosal or parenteral vaccines.

Endnotes

[a] CalfGuard, Zoetis, Parsippany, NJ
[b] Baytril, Bayer Animal Health, Shawnee, KS
[c] SAS/STAT User's Version 9.4, SAS Institute, Cary, NC
[d] Bovi-Shield Gold One Shot, Zoetis, Parsippany, NJ
[e] Dulbecco's Modified Eagle Medium, MilliporeSigma
[f] ACCUSPIN™, Sigma-Aldrich Co. LLC

Acknowledgements

This study was funded by Zoetis Animal Health, Kalamazoo, MI.

References

1. Baker JC. The clinical manifestations of bovine viral diarrhea infection. *Vet Clin North Am Food Anim Pract* 1995;11:425-445.
2. Bellinghausen C, Rohde GGU, Savelkoul PHM, Wouters EFM, Stassen FRM. Viral-bacterial interactions in the respiratory tract. *J Gen Virol* 2016;97:3089-3102.
3. Bergsten C. Causes, risk factors, and prevention of laminitis and related claw lesions. *Acta Veterinaria Scandinavica* 2003;44:S157.
4. Coggins L, Gillespie JH, Robson DS, Thompson JD, Phillips WV, Wagner WC, Baker JA. Attenuation of virus diarrhea virus (strain Oregon C24V) for vaccine purposes. *Cornell Vet* 1961;51:539-545.
5. Corapi WV, French TW, Dubovi EJ. Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhea virus. *J Virol* 1989;63:3934-3943.
6. Cortese VJ, Laim PM. Bovine vaccines and herd vaccination programs. ISVMA 2002:1403-1411.
7. Ellis JA, West KH, Cortese VS, Myers SL, Carman S, Martin KM, Haines DM. Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine virus diarrhea virus-type II. *Can J Vet Res = Revue canadienne de recherche veterinaire* 1998;62:161-169.
8. Fulton RW, Confer A, Burge LJ, Perino LJ, d'Offay JM, Payton ME, Mock RE. Antibody responses by cattle after vaccination with commercial viral vaccines containing bovine herpesvirus-1, bovine viral diarrhea virus, parainfluenza-3 virus, and bovine respiratory syncytial virus immunogens and subsequent revaccination at day 140. *Vaccine* 1995;13:725-733.
9. Fulton RW, Johnson BJ, Briggs RE, Ridpath JF, Saliki JT, Confer AW, Burge LJ, Step DL, Walker DA, Payton ME. Challenge with bovine viral diarrhea virus by exposure to persistently infected calves: Protection by vaccination and negative results of antigen testing in nonvaccinated acutely infected calves. *Can J Vet Res = Revue canadienne de recherche veterinaire* 2006;70:121-127.
10. Fulton RW, Purdy CW, Confer AW, Saliki JT, Loan RW, Briggs RE, Burge LJ. Bovine viral diarrhea viral infections in feeder calves with respiratory disease: Interactions with *Pasteurella* spp, parainfluenza-3 virus, and bovine respiratory syncytial virus. *Can J Vet Res = Revue canadienne de recherche veterinaire* 2000;64:151-159.
11. Fulton RW, Ridpath JF, Confer AW, Saliki JT, Burge LJ, Payton ME. Bovine viral diarrhoea virus antigenic diversity: Impact on disease and vaccination programmes. 2003;31:89-95.
12. Fulton RW, Ridpath JF, Ore S, Confer AW, Saliki JT, Burge LJ, Payton ME. Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. *Vet Microbiol* 2005;111:35-40.
13. Fulton RW, Ridpath JF, Saliki JT, Briggs RE, Confer AW, Burge LJ, Purdy CW, Loan RW, Duff GC, Payton ME. Bovine viral diarrhea virus (BVDV) 1b: Predominant BVDV subtype in calves with respiratory disease. *Can J Vet Res = Revue canadienne de recherche veterinaire* 2002;66:181-190.
14. Fulton RW, Saliki JT, Confer AW, Burge LJ, d'Offay JM, Helman RG, Bolin SR, Ridpath JF, Payton ME. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: Clinical and necropsy samples from cattle. *J Vet Diagn Invest : official publication of the Am Assoc Vet Laboratory Diagnosticians, Inc* 2000;12:33-38.

© Copyright American Association of Bovine Practitioners; open access distribution.

15. Goens SD. The evolution of bovine viral diarrhea: A review. *Can Vet J = La revue veterinaire canadienne* 2002;43:946-954.
16. González AM, Arnaiz I, Yus E, Eiras C, Janjuan M, Dieguez FJ. Evaluation of long-term antibody responses to two inactivated bovine viral diarrhoea virus (BVDV) vaccines. *Vet J* 2014;199:424-428.
17. Griffin D. Economic impact associated with respiratory disease in beef cattle. *Vet Clin North Am Food Anim Pract* 1997;13:367-377.
18. Gunn GJ, Saatkamp HW, Humphry RW, Stott AW. Assessing economic and social pressure for the control of bovine viral diarrhoea virus. *Prev Vet Med* 2005;72:149-162; discussion 215-219.
19. Kapil S, Basaraba RJ. Infectious bovine rhinotracheitis, parainfluenza-3, and respiratory coronavirus. *Vet Clin North Am Food Anim Pract* 1997;13:455-469.
20. Kelling CL. Evolution of bovine viral diarrhea virus vaccines. *Vet Clin North Amer Food Anim Pract* 2004;20:115-129.
21. Klima CL, Zaheer R, Cook SR, Booker CW, Hendrick S, Alexander TW, McAllister TA. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. *J Clin Microbiol* 2014;52:438-448.
22. Liebler-Tenorio EM, Ridpath JE, Neill JD. Distribution of viral antigen and tissue lesions in persistent and acute infection with the homologous strain of noncytopathic bovine viral diarrhea virus. *J Vet Diagn Invest : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 2004;16:388-396.
23. Luzzago C, Bandi C, Bronzo V, Ruffo G, Zecconi A. Distribution pattern of bovine viral diarrhoea virus strains in intensive cattle herds in Italy. *Vet Microbiol* 2001;83:265-274.
24. Mahan SM, Sobecki B, Johnson J, Oien NL, Meinert TR, Verhelle S, Mattern SJ, Bowersock TL, Leyh RD. Efficacy of intranasal vaccination with a multivalent vaccine containing temperature-sensitive modified-live bovine herpesvirus type 1 for protection of seronegative and seropositive calves against respiratory disease. *J Am Vet Med Assoc* 2016;248:1280-1286.
25. Mosier D. Review of BRD pathogenesis: The old and the new. *Animal Health Research Reviews* 2015;15:166-168.
26. Neill JD, Newcomer BW, Marley SD, Ridpath JF, Givens MD. Genetic change in the open reading frame of bovine viral diarrhea virus is introduced more rapidly during the establishment of a single persistent infection than from multiple acute infections. *Virus Res* 2011;158:140-145.
27. Nickell JS, White BJ. Metaphylactic antimicrobial therapy for bovine respiratory disease in stocker and feedlot cattle. *Vet Clin North Am Food Anim Pract* 2010;26:285-301.
28. Nissly RH, Zaman N, Ibrahim PAS, McDaniel K, Lim L, Kiser JN, Bird I, Chothe SK, Bhushan GL, Vandegrift K, Neibergs HL, Kuchipudi SV. Influenza C and D viral load in cattle correlates with bovine respiratory disease (BRD): Emerging role of orthomyxoviruses in the pathogenesis of BRD. *Virology* 2020;551:10-15.
29. Paton DJ, Carlsson U, Lowings JP, Sands JJ, Vilcek S, Alenius S. Identification of herd-specific bovine viral diarrhoea virus isolates from infected cattle and sheep. *Vet Microbiol* 1995;43:283-294.
30. Pellerin C, van den Hurk J, Lecomte J, Tijssen P. Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. *Virology* 1994;203:260-268.
31. Portis E, Lindeman C, Johansen L, Stoltman G. A ten-year (2000–2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex—*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*—in the United States and Canada. *J Vet Diagn Invest* 2012;24:932-944.
32. Richeson JT, Falkner TR. Bovine respiratory disease vaccination: What is the effect of timing? *Vet Clin North Am Food Anim Pract* 2020;36:473-485.
33. Ridpath JF. Bovine viral diarrhea virus: Global status. *Vet Clin North Am Food Anim Pract* 2010;26:105-121.
34. Seeger JT, King ME, Grotelueschen DM, Rogers GM, Stokka GS. Effect of management, marketing, and certified health programs on the sale price of beef calves sold through a livestock video auction service from 1995 through 2009. *J Am Vet Med Assoc* 2011;239:451-466.
35. Spagnuolo M, Kennedy S, Foster J, Moffett DA, Adair BM. Bovine viral diarrhoea virus infection in bone marrow of experimentally infected calves. *J Comp Pathol* 1997;116:97-100.
36. Tautz N, Thiel HJ. Cytopathogenicity of pestiviruses: Cleavage of bovine viral diarrhea virus NS2-3 has to occur at a defined position to allow viral replication. *Archives of Virology* 2003;148:1405-1412.
37. Wang W, Shi X, Wu Y, Li X, Ji Y Meng Q, Zhang S, Wu H. Immunogenicity of an inactivated Chinese bovine viral diarrhea virus 1a (BVDV 1a) vaccine cross protects from BVDV 1b infection in young calves. *Vet Immunol Immunopathol* 2014;160:288-292.
38. Windeyer MC, Gamsjäger L. Vaccinating calves in the face of maternal antibodies: Challenges and opportunities. *Vet Clin North Am Food Anim Pract* 2019;35:557-573.
39. Wood RD, Goens SD, Carman PS, Deregt D, Jefferson B, Jacobs RM. Effect on hematopoietic tissue of experimental infection of calves with noncytopathic type 2 bovine viral diarrhea virus. *Can J Vet Res = Revue canadienne de recherche veterinaire* 2004;68:42-48.
40. Woolums AR, Siger L, Johnson S, Gallo G, Conlon J. Rapid onset of protection following vaccination of calves with multivalent vaccines containing modified-live or modified-live and killed BHV-1 is associated with virus-specific interferon gamma production. *Vaccine* 2003;21:1158-1164.
41. Xue W, Ellis J, Mattick D, Smith L, Brady R, Trigo E. Immunogenicity of a modified-live virus vaccine against bovine viral diarrhea virus types 1 and 2, infectious bovine rhinotracheitis virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus when administered intranasally in young calves. *Vaccine* 2010;28:3784-3792.
42. Xue W, Mattick D, Smith L, Umbaugh J, Trigo E. Vaccination with a modified-live bovine viral diarrhea virus (BVDV) type 1a vaccine completely protected calves against challenge with BVDV type 1b strains. *Vaccine* 2010;29:70-76.

© Copyright American Association of Bovine Practitioners; open access distribution.