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Efficacy of a modified-live IBR-BVD-PI3-BRSV-*Mannheimia haemolytica* toxoid vaccine against challenge with virulent BHV-1 and PI3 viruses in calves 60 days of age

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Abstract

Efficacy of attenuated *(att)* bovine herpesvirus type 1 (BHV-1) and bovine parainfluenza type-3 (PI3) viruses as antigen fractions in a modified-live virus (MLV) multivalent vaccine was evaluated following single, subcutaneous (SC) administration and intranasal (IN) challenge 28 days after vaccination, with either virulent BHV-1 or PI3 viruses in young calves. A total of 80 seronegative calves, 50 to 63 days of age at the time of vaccination, were used in 2 separate studies with 40 animals per study. Calves were allocated to 2 treatment groups with 20 animals per group which received either a single dose of a MLV IBR-BVD-PI3-BRSV vaccine, *Mannheimia haemolytica* toxoid, or corresponding placebo formulation without targeted test antigen fractions *att*BHV-1 (Study 1) or *att*PI3 (Study 2). Incidence and duration of clinical signs associated with respiratory disease, rectal temperatures, virus shedding, and serologic responses were compared between treatment groups in each study to assess vaccine efficacy. The 5-way vaccine induced significantly higher ($p<0.0001$) virus neutralizing antibody responses and reduction in fever ($p<0.0001$), mean rectal temperatures, and lower incidence or shorter duration of clinical disease related to BHV-1 and PI3 infection than placebo-treated calves. Infectious bovine rhinotracheitis (IBR) disease, hallmark of BHV-1 infection, was observed in 95% of control calves and in only 10% of vaccinates. Vaccinated animals in Study 1 and Study 2 demonstrated a 98.8 and 98.9% reduction in virus shedding, respectively, and significantly ($p<0.0001$) shorter duration of virus shedding compared to control calves, demonstrating protective vaccine efficacy.

Key words: bovine, BHV-1, BRD, PI3, toxoid, 5-way viral vaccine

Introduction

Bovine respiratory disease (BRD) is the most common disease in beef and dairy cattle worldwide, and a major cause of significant economic losses.[14] Unvaccinated calves are highly susceptible to infections with multiple viral and bacterial pathogens associated with BRD.[15] Viral pathogens such as bovine herpes virus type-1 (BHV-1), bovine parainfluenza type-3 virus (PI3), bovine viral diarrhea virus type 1 and 2 (BVDV), and bovine respiratory syncytial virus (BRSV) are recognized as either contributing or primary etiological agents in BRD.[20] These viral pathogens cause significant damage to epithelium of the upper and lower respiratory tract and are associated with shipping fever in cattle transported to feedlots.[11,12]

BHV-1 is the causative agent of infectious bovine rhinotracheitis (IBR), a contagious respiratory disease affecting cattle of all ages. BHV-1 infections are often linked to immune suppression, conjunctivitis, encephalitis, abortion, and generalized systemic infection.[14,18] PI3 is a recognized BRD pathogen that persists endemically in dairy and beef herds.[9,17] Clinical disease related to PI3 infections is commonly found in calves with failure of passive transfer or insufficient colostrum intake or with rapid decay of maternally derived antibodies. Clinical representation of PI3 in the field is often mild, consisting of occasional fever, nasal discharge, and dry cough.[9,17] PI3 infection is commonly complicated by co-infection with other BRD pathogens and is therefore an important component of enzootic pneumonia in calves and BRD in feedlot cattle, contributing to substantial economic losses. Efficacy in controlling BRD related to viral infections depends on the combination of control measures, improved herd management practices, and vaccination.[1,14,29] Combined with good management practices, vaccination is considered the most effective method for management of BRD.[7] Several

modified-live viral (MLV) and killed viral and bacterial vaccines are available commercially. Their efficacy in reducing the morbidity and mortality in calves due to BRD, including BHV-1 and PI3 pathogens, has been demonstrated.[7,10,23,34,35] The timing of vaccine administration against different BRD antigens is key for vaccine efficacy and BRD prevention.[29] In North America key animal handling time periods, such as shortly after birth (neonatal calves), during branding (nursing calves 60 to 120 d of age), at or around weaning (~205 d of age), and on arrival at stocker, backgrounder and/or feedlot facilities are often used to vaccinate cattle.[29] Presented in this report are the results from 2 separate studies which aimed to evaluate the efficacy of *att*BHV-1 and *att*PI3 fractions from a multivalent, MLV vaccine-toxoid in calves 60 d of age following a single subcutaneous (SC) administration and challenge with virulent BHV-1 and PI3 viruses at 28 d post-vaccination.

Materials and Methods

Animals and Housing

Neonatal, day-old, Holstein and Holstein-cross, colostrum-deprived calves were sourced from a commercial dairy. Calves were sourced on 2 occasions (40 animals each time) for the purposes of 2 separate studies, and shipped to a Zoetis research farm (Richland, MI) where they were raised until approximately 60 d of age (60 to 63, Study 1; and 53 to 60, Study 2). Newborn calves were housed in individual pens and fed electrolytes until 48 h of age to avoid ingestion of maternal antibodies, after which they received 3 quarts (2.8 l) of milk replacer twice daily, every 12 h until 6 weeks (wk) of age; calves were gradually weaned off milk replacer by introducing sweetfeed around 3 weeks of age; thereafter, a commercial high quality starter diet with ration that met or exceeded nutritional requirements for the age of animals was available free-choice. At birth, animals were vaccinated against enteric rotavirus and coronavirus,[a] and no other treatments were administered prior to the beginning of the study. During the vaccination phase, animals in both studies were housed individually and allotted by treatment in 2 identical rooms in a BSL-2 facility in order to prevent exposure of control calves to viruses shed from vaccinated animals. At the time of viral challenge, vaccinated and control calves in each study were commingled, divided into 2 group-housed rooms in the BSL-2 facility. All study protocols were reviewed and approved by the Zoetis Institutional Care and Use Committee before the start of the study.

Experimental Design and Randomization

Two separate, randomized, controlled studies with 40 calves per study were conducted to evaluate the efficacy of the *att*BHV-1 and *att*PI3 fractions in a MLV-toxoid vaccine administered as a single SC dose for protection of young calves, ~ 60 d of age, against virulent BHV-1 or PI3 challenge. For each study, there were 2 treatment groups (vaccinated or placebo), 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. During the vaccination phase, room was the experimental unit, while the animal was the experimental unit during the challenge phase of the study. Animals were allocated to treatments, vaccination phase rooms, and challenge rooms per the randomization plan generated by a Zoetis biometrics representative. The random treatment allocation plan was created using a commercial statistical program[b] 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 BHV-1 and PI3 (serum VN antibody titer against BHV-1 and PI3 of < 1:2 on day of vaccination).

Vaccination and Challenge

Calves in both study groups were administered a combination MLV BHV-1, BVDV, PIV3, BRSV vaccine + *Mannheimia haemolytica* toxoid, while animals in the placebo-control group received placebo formulated in the same way but without the *att*BHV-1 (Study 1) or *att*PI3 (Study 2) fractions. The respective vaccine fractions were titrated for an input level below the established minimum immunizing dose (MID) of the commercial product.[c] 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 28 post-vaccination, individual calves were challenged IN with either virulent BHV-1 (Cooper strain; 7.7 $\log_{10}$ $TCID_{50}$/4 mL dose; Study 1) or PI3 strain (8.17 $\log_{10}$ $TCID_{50}$/4 mL dose; Study 2). Both challenge strains were

Table 1. Summary of study design in calves experimentally challenged with BHV-1 (Study 1) or PI3 (Study 2) after vaccination with a multivalent 5-way vaccine or placebo.

Challenge		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	
Virus	Strain		Vaccine	Placebo			Challenge	Completion
BHV-1	Cooper	Intranasal	20	20	7.7 $\log_{10}$ 4 mL	60-63	28	42
PI3V	NVSL	Intranasal	20	20	8.17 $\log_{10}$ 4 mL	57-60	28	42

obtained from The National Veterinary Services Laboratories (NVSL), Ames, Iowa. A compressed gas atomizer was used to administer the 4 mL dose (2 mL per nostril) of the challenge material to each calf. At the end of each study, all animals were humanely euthanized in compliance with the American Veterinary Medical Association Guidelines for Humane Euthanasia[d] and disposed by secure burial.

Clinical Assessment

Following challenge with virulent BHV-1 or PI3, calves were monitored for 14 consecutive days for presence of fever (≥104.0°F; ≥40°C) and clinical signs of respiratory disease (depression, dyspnea, cough, and nasal discharge). 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.

In Study 1, animals in each treatment group were diagnosed with IBR disease if they developed pyrexia (≥104.0°F; ≥40°C) for at least 2 days, and demonstrated depression, respiratory effort (dyspnea), and/or nasal discharge (scores of ≥1) at any point during the post-challenge observation period. Frequency of individual clinical signs, including pyrexia, within each treatment group, were compared and analyzed.

In Study 2, due to the known lower virulence of the PI3 NVSL challenge virus resulting in mild clinical signs, vaccine efficacy was determined by comparing and analyzing virus shedding titers, and the duration of virus shedding from nasal secretions post-challenge.

Table 2. Clinical scoring system for BHV-1 and PI3 challenge phase clinical evaluation.

	Clinical Score
Depression	0 = Normal 1 = Abnormal. Animal tends to stand with head lower than normal. Has a dull appearance in 1 or both eyes; 1 or both ears may droop lower than ears of roommates. Animal is lethargic with movements and responses to stimuli that are slow, hesitant or unsteady. Animal has a reduced interest in surroundings and may stand off from roommates or from feed. If recumbent, animal is markedly slower in rising and rises (maybe unsteadily) with increased effort.
Respiratory effort (dyspnea)	0 = Normal 1 = Abnormal. Respiratory character may be deep, and primarily abdominal or markedly shallow and rapid. Breathing may be audible as raspy or with an expiratory "grunt" during exhalation.
Nasal discharge	0 = Normal 1 = Normal. Small to notable amount of serous discharge accumulated in or draining from nostrils. 2 = Abnormal. Notable amount (approximately ≥ 5 mL) of persistent mucopurulent discharge accumulated in or draining from nostrils.

Laboratory Analysis

To evaluate virus shedding from the upper respiratory tract post-challenge, nasal swabs were collected from each animal prior to challenge and each day after challenge (d 29 to 42) in both studies. A single sterile swab was used to swab a single naris of each animal on designated collection dates. The swabs were placed into a vial with minimum essential media containing an antibiotic and antimycotic,[e] and held on wet ice during collection and stored at -94 ±50°F (-70 ±10°C) until tested as previously described.[23] A whole blood sample was collected from a jugular vein of each calf prior to vaccination (d -1), prior to viral challenge (d 27), and on d 42 (end of study). Serum was harvested and stored at 39.2°F (4°C) until virus neutralization (VN) testing, as previously described.[23]

Statistical Analysis

A designated veterinary medicine research and development biometrician was responsible for data summaries and analyses of data entered into the centralized data management system.[b] The room housing each study group was the experimental unit relating to the vaccination phase of the study, while calf was the experimental unit during the viral challenge phase. 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 virus shedding was determined for each animal, and was calculated as the last time point present minus the first time point present, plus 1. Duration of virus shedding was set to zero for animals that had no time points with positive BHV-1 and PI3 virus isolation. Duration of virus shed 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 virus isolation collection time point. Durations were subsequently compared between treatment and control groups in each experiment with a general linear mixed model, with treatment as a fixed effect. Challenge room and the residuals were treated as random effects. Data on virus shedding, challenge-phase rectal temperatures, and challenge phase antibody titers were each compared between groups within each study with a general linear mixed model with repeated measures. Treatment, assessment point, and the interaction between these 2 variables were fixed effects. Challenge room, individual calf within challenge room, and residuals were random effects. Least squares means (LSM) values of $P \leq 0.05$ were considered significant for all analyses.

Results

Vaccine Safety and Animal Removal

Eighty calves (40/study), were vaccinated at ~60 d of age with either the 5-way MLV + *M. haemolytica* toxoid (20/study), or placebo vaccine without *att*BHV-1 (Study 1; 20

calves) or without *att*PI3 (Study 2; 20 calves). None of the animals in either study showed signs of an adverse reaction, such as injection site reactions, fever, anaphylaxis or tremor following the vaccine or placebo. Two animals from the placebo group in Study 2 (PI3 efficacy) were removed for health reasons (lameness and unthrifty), unrelated to vaccination. One animal was removed 11 d after vaccination while the other calf was removed 2 d post-challenge (study d 30).

Respiratory Disease

Study 1 (attBHV-1 efficacy). The *att*BHV-1 vaccine fraction induced significant protection against BHV-1 respiratory disease. Following challenge with virulent BHV-1, 19 of 20 (95%) control calves developed signs of IBR, whereas only 2 of 20 (10%) of vaccinated calves showed signs of IBR (p=0.0002) (Table 3).

Incidence and duration of clinical signs associated with BHV-1 infection (pyrexia, depression, nasal discharge, and respiratory effort) following BHV-1 challenge were analyzed. The incidence of pyrexia differed (P < 0.0001) between groups, with control calves (20 of 20) developing pyrexia (≥104.0°F; ≥40°C) following challenge while 4 of 20 (20%) vaccinated calves were febrile (Table 3). Furthermore, on study d 30 through d 36 (d 2 to 8 post-challenge) there was a significant reduction in mean rectal temperatures (Figure 1A) and the percent of febrile animals in the vaccinated group (0%, 10%, 10%, 5%, 5%, 0%) compared to placebo group (35%, 95%,100%, 100%, 60%, 50%) (Table 3). The mean duration of pyrexia in vaccinated calves was reduced (p<0.0001) compared to placebo calves (0.3 d vs 4.8 d). Onset of fever in the placebo group occurred 2 d post-challenge (Table 3), with peak fever occurring on study d 32 and 33 (d 4 to 5 post-challenge) when all 20 calves were febrile (≥104.0°F; ≥40°C). Only 2 vaccinated calves had fever during those days (Table 3).

Increased respiratory effort was observed in 30 (6/20), 35 (7/20), and 30% (6/20) of calves in the placebo group on study d 35, 36, and 37 (d 7 to 9 post-challenge), respectively, compared to 0 (0/20), 5 (1/20), and 0% (0/20) in the vaccinated calves (p=0.02, p=0.04, and p=0.02, respectively (Table 3), on the same days.

Ninety percent of vaccinates and 95% of controls had abnormal nasal discharge at least once following viral challenge. Fewer calves in the vaccinated group had nasal discharge compared to calves in the placebo group. Particularly, on study d 36 and 37 (d 8 and 9 post-challenge; Table 3), 65 (13/20) and 70% (14/20) of control calves had abnormal nasal discharge compared to only 25% in the vaccinated group (p=0.02 and p=0.01, respectively; Table 3).

Study 2 (attBPI3 efficacy) Since the NVSL PI3 virus is low virulence, there were no noticeable respiratory clinical signs at any point following challenge with the NVSL PI3 virus (Table 4). Five of 18 (28%) calves in the placebo had fever on either a single occasion (2 animals, study d 32 and 33), 2 consecutive days (2 calves on d 32-33 and 41-42) or 3 consecutive days (1 calf; d 40-42). In contrast, only 2 animals in the vaccinated group showed signs of fever; 1 calf was febrile on d 32 and another calf was febrile on study d 38 and 39 (Table 4). There was a significant difference (p=0.03) in mean rectal temperatures between the vaccinates and placebo-

Table 3. Percent of animals displaying clinical signs of BHV-1 respiratory disease and virus shedding following challenge.

	Percentage (%) of animals by day after challenge*															
Study day	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Day post-challenge	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fever																
Control calves†	0	0	0	35	95	100	100	60	50	5	0	0	5	5	5	0
Vaccinated calves†	0	0	0	0[a]	10[c]	10[c]	5[c]	5[c]	0[c]	0	0	0	0	0	0	0
Respiratory effort																
Control calves	0	0	0	0	0	0	0	15	30	35	30	15	20	5	5	0
Vaccinated calves	0	0	0	0	0	0	0	0	0[a]	5[a]	0[a]	0	0	0	0	0
Depression																
Control calves	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
Vaccinated calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nasal discharge																
Control calves	0	0	0	0	5	10	15	45	60	65	70	55	45	60	45	40
Vaccinated calves	0	0	0	0	10	10	20	40	25	25[a]	25[a]	35	40	30	20	30
BHV-1 virus shedding																
Control calves	0	0	100	100	100	100	100	100	100	100	100	95	95	85	65	40
Vaccinated calves	0	0	90	100	100	100	90	90	70[a]	20[c]	30[c]	25[c]	5[c]	10[c]	5[c]	0[b]

*Values differ if p<0.05[a], p<0.001[b], and p<0.0001[c]

†Controls vaccinated with combination MLV BVDV, BRSV, plus *Mannheimia haemolytica* vaccine. Vaccinates vaccinated with combination BHV-1, BVDV, BRSV, PI3V, *Mannheimia haemolytica* vaccine (Bovi-Shield Gold® 5 + OneShot, Zoetis, Parsippany, NJ)

treated calves (Figure 1B) observed on study d 32, 34, and 42 (d 4, 5, and 14 post-challenge), which correlates with fever spikes after challenge (Table 4). There was no difference in the number of calves demonstrating respiratory effort, depression or nasal discharge between the 2 groups (Table 4).

Virus Shedding

Vaccine efficacy in both studies was assessed by comparing the load and duration of virus shedding following challenge with either BHV-1 or PI3 viruses. All calves were negative for BHV-1 (Study 1) or PI3 (Study 2) virus before the challenge phase as determined by virus isolation (VI).

Study 1. *BHV-1 Shedding.* All placebo and vaccine-treated calves (40/40) shed BHV-1 from nasal secretions following IN challenge (Table 3). From d 29 through d 41 (challenge phase), vaccinated calves showed a significant reduction in the amount of virus shed compared to placebo-treated calves (Figure 2A). From d 35 to the end of the study

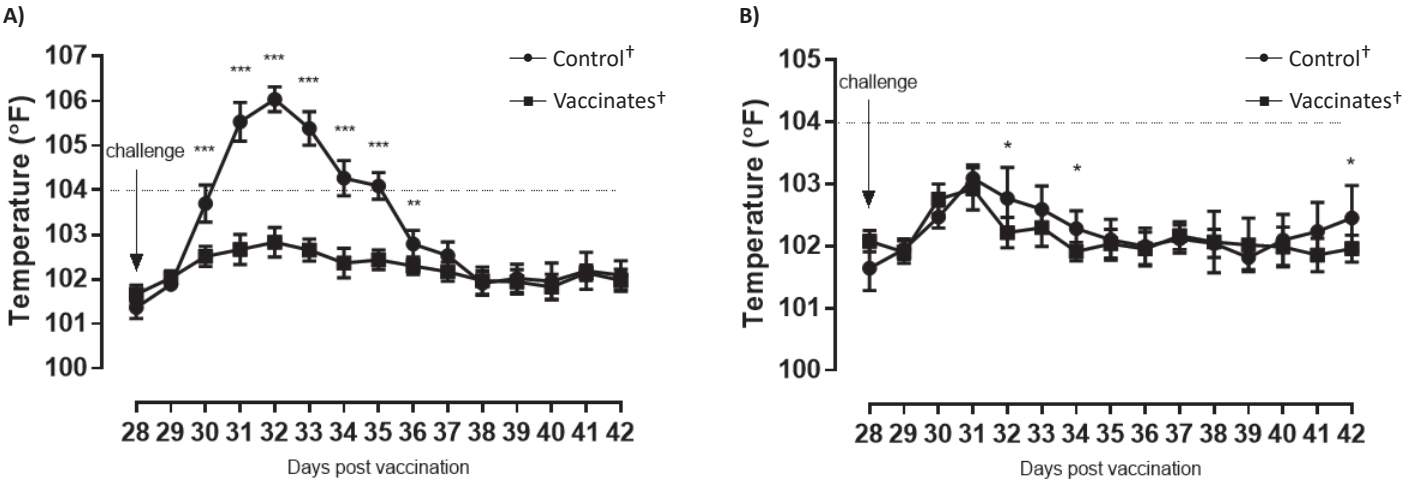

Figure 1. Least squares means (LSM) of rectal temperatures in calves following vaccination with multivalent 5-way MLV vaccine** and challenge with virulent BHV-1 (A) or PI3 (B).

*Data points significantly different if p<0.0001(***), p<0.001 (**), and p<0.05 (*)

†Controls vaccinated with combination MLV BVDV, BRSV, plus *Mannheimia haemolytica* vaccine. Vaccinates vaccinated with combination BHV-1, BVDV, BRSV, PI3V, *Mannheimia haemolytica* vaccine (Bovi-Shield Gold® 5 + OneShot, Zoetis, Parsippany, NJ)

Table 4. Percent of animals displaying clinical signs of PI3 respiratory disease and virus shedding following challenge.

	Percentage (%) of animals by day after challenge														
Study day	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Day post-challenge	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fever															
Control calves*	0	0	0	5.6	11.1	5.6	0	0	0	0	0	0	5.6	5.6	5,6
Vaccinated calves*	0	0	0	5	0	0	0	0	0	0	5	5	0	0	0
Respiratory effort															
Control calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaccinated calves	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
Depression															
Control calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaccinated calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough															
Control calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaccinated calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nasal discharge															
Control calves	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaccinated calves	0	0	0	5	5	5	0	0	0	0	0	0	0	0	0
PI3 virus shedding															
Control calves	0	100	100	100	100	100	100	100	88.9	44.4	22.2	16.7	5.6	0	0
Vaccinated calves	0	100	100	100	100	85	25[c]	15[c]	5[c]	0[b]	0[a]	0	0	0	0

*Data points significantly different if p<0.05 (a); p<0.001 (b) and p<0.0001(c)

(d 42), percentage of animals shedding virus in the vaccinated group was 70%, 20%, 30%, 25%, 5%, 10%, 5%, and 0% compared to calves in the placebo group; 100%, 100%, 100%, 95%, 95%, 85%, 65%, and 40%, respectively. The differences were significantly different at each time point (Table 3). Based on the area under the curve (total virus shed), there was a 98.8% reduction in geometric LSM virus titers shed by vaccinates compared to controls during the post-challenge period ($7.3x10^5$ geometric LSM vaccinated group vs $6.2x10^7$ geometric LSM placebo group; ($p<0.0001$; Table 5A). The duration of virus shedding following challenge was shorter in vaccinated calves (8 days) compared to placebo calves (13.1 days; $p<0.0001$), suggesting a strong protective vaccine effect (Table 5A).

Study 2. *PI3 Shedding.* Similar to Study 1, all placebo and vaccinated calves (40/40) had shed detectable PI3 virus from their nasal passages following IN challenge (Table 4). Significant reduction in the daily amount of virus shed post-challenge was observed from d 29 to d 36 in the vaccinated group compared to controls (Figure 2B). Furthermore, there were significantly fewer vaccinated animals shedding PI3 virus from study d 34 until d 38 (25%, 15%, 5%, 0%, 0%, respectively), compared to calves in the placebo group (100%, 100%, 88.9%, 44.4%, and 22.2% on the same days; Table 4). Area under the curve analysis revealed that vaccine induced a 98.9% reduction ($p<0.0001$) in PI3 virus shedding; geometric LSM virus titers shed in the vaccinated calves were $6.05x10^4$ compared to $5.57x10^6$ in placebo calves (Table 5B). Consistent with the vaccine effect described for the BHV-1 vaccination study, the duration of PI3 virus shedding was significantly reduced ($p<0.0001$) in vaccinated calves (5.4 d) compared to calves in the placebo group (9.4 d) (Figure 2B; Table 5B).

Serology

All animals were seronegative (VN titer <1:2) to BHV-1 or PI3 prior the start of the studies. Control placebo-treated animals remained seronegative prior to challenge (d 27).

Study 1. At 27 d following challenge with virulent BHV-1, 20 of 20 vaccinates had seroconverted (titer titer >1:2). The LSM virus neutralizing (VN) titers of vaccinated calves were 25±3.5 and were significantly higher ($p<0.0001$) than the LSM titers in control calves (LSM titer of 1±0.3; Figure 3A). Following viral challenge, all calves in the vaccinated group demonstrated evidence of anamnestic response. The LSM of VN antibody titers in vaccine-treated calves increased from 25±3.5 to 140 ±18.6, which was higher ($p<0.0001$) than the VN LSM titer of 35±2.2 observed in control calves at the end of the study.

Study 2. Vaccination with MLV vaccine containing *att*PI3 fraction induced seroconversion in 15 of 20 animals (titer >1:2) by d 27, and the LSM VN serum antibody titer against PI3 in vaccinated calves was higher compared to control calves (LSM of 11±2.6 vs LSM of 1±0.5; $p<0.0001$; Figure 3B). On d 42, the PI3 VN LSM titer of the vaccinated group increased to 1470±331.2 compared to controls (LSM antibody titer of 66±5.3; $p<0.0001$). The differences in VN titer responses following vaccination and challenge demonstrate protective efficacy of the *att*PI3 vaccine fraction.

Discussion

Bovine respiratory disease (BRD) is a complex disease resulting from multiple factors, such as stress-induced immunosuppression, infection with 1 or more viruses, and often followed by bronchopneumonia caused by commensal

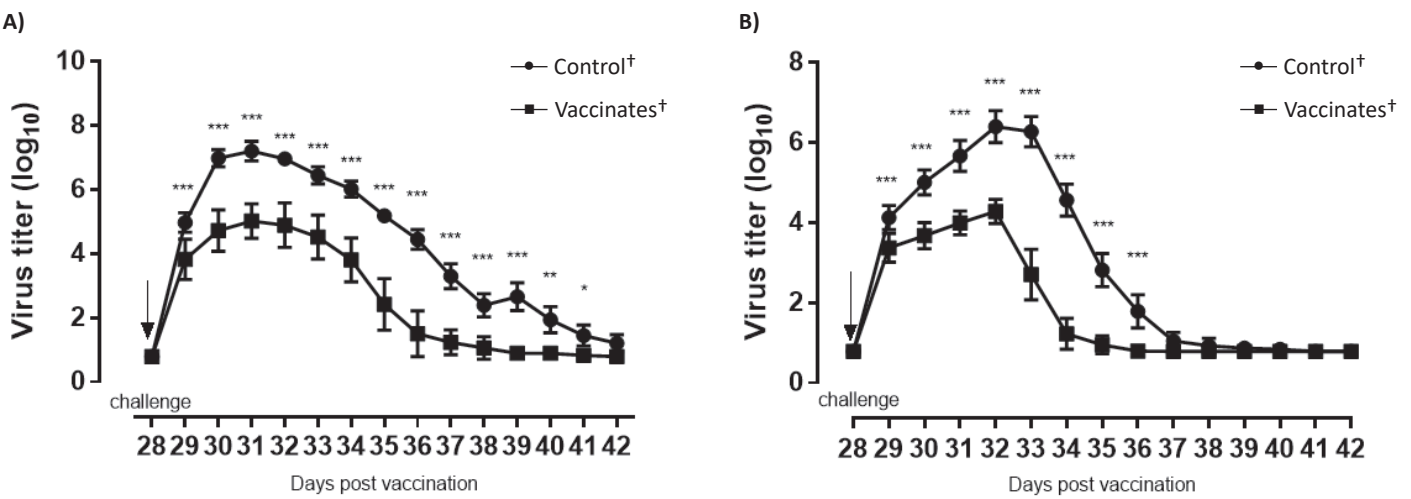

Figure 2. LSM of BHV-1 virus Study 1 (A) and PI3 virus Study 2 (B) titer in nasal secretion samples collected from the calves following challenge with virulent (A) BHV-1 or (B) PI3.

*Data points significantly different if $p<0.0001$(***), $p<0.001$ (**), and $p<0.05$ (*)

†Controls vaccinated with combination MLV BVDV, BRSV, plus *Mannheimia haemolytica* vaccine. Vaccinates vaccinated with combination BHV-1, BVDV, BRSV, PI3V, *Mannheimia haemolytica* vaccine (Bovi-Shield Gold® 5 + OneShot, Zoetis, Parsippany, NJ)

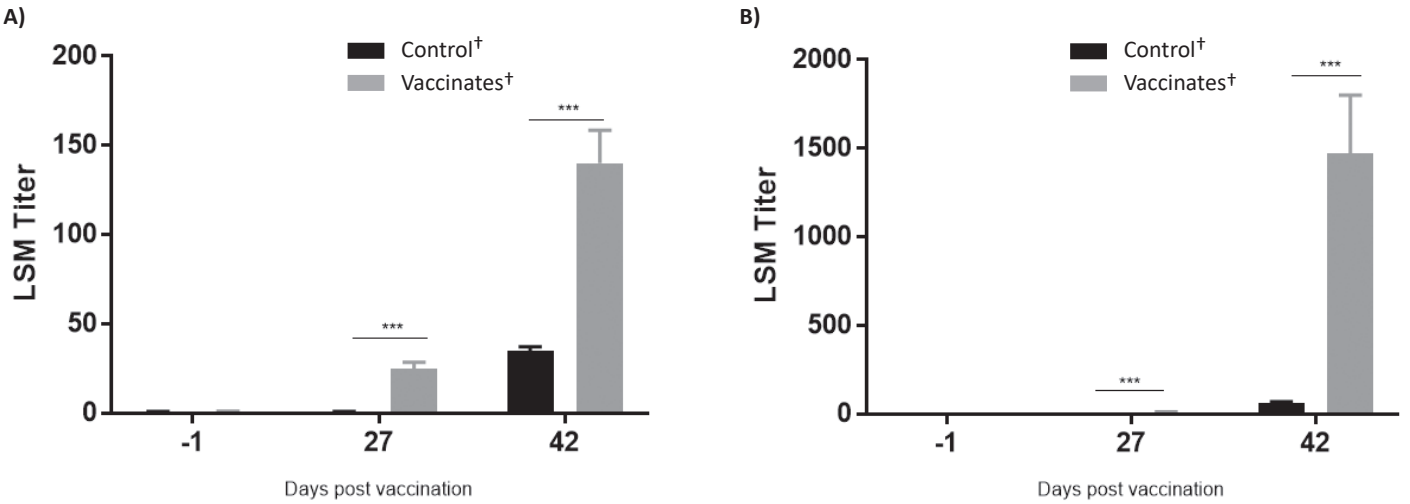

Figure 3. LSM of virus neutralizing serum antibody titers against (A) BHV-1 (Study 1) and (B) PI3 (Study 2), at pre-vaccination (day -1), post-vaccination (day 27) and post-challenge (day 42).
*Values differ if p<0.0001(***), p<0.001 (**), and p<0.05 (*)
†Controls vaccinated with combination MLV BVDV, BRSV, plus *Mannheimia haemolytica* vaccine. Vaccinates vaccinated with combination BHV-1, BVDV, BRSV, PI3V, *Mannheimia haemolytica* vaccine (Bovi-Shield Gold® 5 + OneShot, Zoetis, Parsippany, NJ)

Table 5. Summary of incidence and duration of (**A**) BHV-1 (Study 1) and (**B**) PI3 virus shedding (Study 2) for 2 experimental groups challenged with BHV-1 or PI3 virus.*

A)			
	Control†	**Vaccinates†**	**P value**
IBR disease	95%	10%	p<0.0002
Duration of shedding (days)	13.1	8	p<0.0001
Virus load (Geometric LSM)	$6.2x10^7$	$7.3x10^5$	p<0.0001
Reduction in BHV-1 shedding	98.8%		
B)			
	Control	**Vaccinates**	**P value**
Duration of shedding (days)	9.4	5.4	p<0.0001
Virus load (Geometric LSM)	$5.57 x10^6$	$6.05x10^4$	p<0.0001
Reduction in PI3 virus shedding	98.9%		

*Values differ if $p<0.05^a$, $p<0.001^b$, and $p<0.0001^c$
†Controls vaccinated with combination MLV BVDV, BRSV, plus *Mannheimia haemolytica* vaccine. Vaccinates vaccinated with combination BHV-1, BVDV, BRSV, PI3V, *Mannheimia haemolytica* vaccine (Bovi-Shield Gold® 5 + OneShot, Zoetis, Parsippany, NJ)

bacteria present in the nasopharynx. High to moderate morbidity, mortality, low feed conversion, reduced average daily gain, and antibiotic or supportive treatments contribute to the significant economic losses in both the beef and dairy industries. Several viruses, such as BHV-1, PI3, BRSV, and BVDV, play contributing roles in the pathogenesis of BRD as primary or confounding factors in disease occurrence.[20] To date, vaccination remains 1 of the most widely used and most effective preventative measures against BRD in both dairy and beef operations.[7] Proper timing of vaccination is of paramount importance for success of vaccine efficacy and disease control.[29] The earliest opportunity to vaccinate beef and dairy calves is at birth; however, this time point is often challenging both logistically and immunologically.[29,33] Preconditioning is a longstanding management practice that can be implemented to improve subsequent health and performance in beef cattle.[29,30] This practice includes a series of vaccination and management schemes at various age stages in order to better prepare calves for their transition to stocker and feeder sectors of the industry.[30]

The purpose of the current study was to explore whether a MLV 5-way vaccine could confer protection against experimentally induced BHV-1 and PI3 infection in young calves at ~ 60 d of age following single SC vaccination. This age often coincides with branding or summer turn-out on beef operations, and can be a time for primary or booster vaccination.

Bovine herpesvirus-1 is a well-known causative agent of BRD in cattle, either as the primary disease agent or in

combination with bacterial pathogens such as *M. haemolytica*.[19,36,37] Uncomplicated BHV-1 infection is characterized by low mortality, high morbidity, abrupt and high fever (≥104 °F; ≥40 °C), conjunctivitis, profound nasal discharge, rhinitis, tracheitis, and in some rare cases encephalitis and abortion, resulting in substantial economic losses to the cattle industry. Vaccines that can be administered by the intramuscular (IM), SC, or IN route are available, and their efficacy in protection from disease following challenge has been established.[16,21,22,28,31] Vaccination by the IM or IN routes can induce rapid protection against experimental challenge.[16,21,22,28] Although the onset of immunity can be as short as 5 to 7 days following vaccination with BHV-1 vaccine, the goal of this study was to use a standard challenge model with challenge on d 28 post-vaccination for both BHVI and PI3 studies. In the current study, single SC vaccination with combination MLV BHV-1-BVDV-BPI3-BRSV+*M. haemolytica* toxoid vaccine induced clinically relevant protection against BHV-1 challenge. Rectal temperature and 3 clinical signs were monitored: respiratory effort (dyspnea and or tachypnea), depression, and nasal discharge. These signs are typically induced by the Cooper strain challenge virus, but are also commonly seen in natural cases of IBR. The frequency and incidence of 2 of 3 signs (no depression) and fever were consistently observed in the control calves compared to the vaccinates. In addition, comparing the study definition of IBR disease (2 d of fever plus 1 of 3 clinical signs), only 10% of the vaccinates were scored with IBR whereas 95% of control calves were scored positive, demonstrating that the vaccine induced protection against challenge dose. Significant reduction in the percentage of calves shedding the virus, daily and total virus load, and overall duration of shedding suggested protective immunity was conferred by single vaccination with the MLV + MH vaccine in 60 d-old calves. Protection of vaccinates against BHV-1 challenge was correlated with development of virus neutralization titers pre-challenge, with an increase in titers after challenge. These results were consistent with those of a previous study[13] in which similar amounts of anti–BHV-1 antibodies were observed after parenteral vaccination.[13]

Bovine PI3 virus is an endemic virus circulating in dairy and beef cattle around the world since the 1960s.[9] Since then, PI3 has been recognized and reported in field cases as a contributor to BRD with a variety of clinical signs and severity.[2,3,4,6,9] The complex nature of BRD and the involvement of multiple pathogens in its etiology make it difficult to ascertain the role of PI3 in BRD. Attempts to create clinical BRD by intranasal or intratracheal inoculation, aerosol delivery or combinations of these routes of inoculations were done several years ago, and mostly provided mixed results ranging from asymptomatic infection to severe pneumonia.[5,8,27] Most uncomplicated PI3 infections are mild with rare cough, nasal discharge, and transient fever with most animals recovering in a few (≤10) days.[9] In the current study, challenging calves with a high virus load resulted in an asymptomatic infection in control calves that was characterized by intermittent fever and excessive virus shedding without clinical signs (Table 3). The PI3 challenge strain used in this study is recognized by the Center of Veterinary Biologics (CVB) as a reference strain for challenge purposes for vaccine licensure, and has a known low clinical presentation. Even with the asymptomatic infection, intermittent fever, and duration and load of virus shedding in the placebo-treated calves (9.4 days), the outcome was comparable to observations from field and other experimental studies[4,8] and confirmed successful challenge procedures. Protection from PI3 infection is mediated by mucosal and serum VN antibodies after exposure to live virus, as well as a reduction in amount and duration of virus shed through nasal secretions.[24,26] In Study 2, a single SC vaccination with MLV vaccine induced seroconversion by d 28 in 15 of 20 vaccine-treated calves by the time of challenge, with LSM titers of VN antibodies reaching value 11. It is well documented that re-exposure to PI3 commonly results in accelerated anamnestic serum and mucosal antibody responses, reaching significantly higher neutralizing antibody titers.[24] Consistent with this observation, there was an anamnestic VN antibody response ($p<0.0001$) in Study 2 following secondary exposure to PI3 antigen, suggesting adequate priming and memory response following vaccination (Figure 3B). The high VN antibody titers observed post-challenge (LSM 1470) correlated with a significant decrease in the number of calves shedding the virus, duration of shedding, and virus load resulting in overall 98% reduction of shedding in vaccinated calves compared to controls. In comparison, the control calves mounted a primary immune response to PI3 and achieved a LSM titer of 66. Self-limiting virus shedding is a hallmark of the PI3 disease model (and in natural infection), regardless of the immune status of the animal, and cannot be prevented. Protective immunity to PI3 is due to both humoral and cell-mediated immune responses, and relies on recall immune responses in vaccinated or previously exposed animals. Although limited information about the role of cell-mediated immune response to PI3 infection in cattle[9,32] exists, a lot more is known about the role of antibody responses. Titers of 1:32 are considered to be protective, and colostrum-fed calves were still susceptible to infection but are spared from severe effects.[9] In this study, because the existing vaccine primed immunity, vaccinated calves shed less virus, the peak levels of virus shedding were lower, and duration was significantly shorter compared to the placebo group.

Conclusion

Under the conditions of this study, a single SC dose of multivalent, MLV vaccine modelled on a commercial vaccine was safe. No adverse effects were associated with vaccine administration to 60 d-old Holstein and Holstein-cross calves. A single dose of the vaccine induced clinically relevant, disease-sparing protective immunity against BHV-1 and PI3 respiratory challenge in naïve 60 d-old calves. This age is linked to branding or turn-out activities, and is a convenient time to vaccinate calves, thereby building immunity against key pathogens in advance of weaning. Additional research is needed to better characterize the duration of immunity,

cell-mediated component of the immune responses, and the potential to extend or improve the response by re-vaccination with additional vaccination.

Endnotes

[a] CalfGuard®, Zoetis, Parsippany, NJ
[b] SAS/STAT User's Version 9.4, SAS Institute, Cary, NC
[c] Bovi-Shield Gold One Shot® Zoetis, Inc., Parsippany, NJ
[d] Available at: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf
[e] Anti-Anti 100X, Gibco®, Thermo Fisher Scientific, Waltham, MA

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