

Effect of calf age and administration route of initial multivalent modified-live virus vaccine on humoral and cell-mediated immune responses following subsequent administration of a booster vaccination at weaning in beef calves

Amelia R. Woolums, DVM, PhD; Roy D. Berghaus, DVM, PhD; Londa J. Berghaus, MS; Roger W. Ellis, DVM, MS; Mel E. Pence, DVM, MS; Jeremiah T. Saliki, DVM, PhD; Katherine A. E. Hurley, BA; Kimberly L. Galland, BS; William W. Burdett, DVM; Scott T. Nordstrom, DVM; David J. Hurley, PhD

Objective—To compare immune responses following modified-live virus (MLV) vaccination at weaning after intranasal or SC administration of an MLV vaccine to beef calves at 2 or 70 days of age.

Animals—184 calves.

Procedures—Calves were allocated to 1 of 5 groups. The IN2 (n = 37) and IN70 (37) groups received an MLV vaccine containing bovine herpesvirus 1 (BHV1), bovine viral diarrhea virus (BVDV) types 1 and 2, bovine respiratory syncytial virus (BRSV), and parainfluenza 3 virus intranasally and a *Mannheimia haemolytica* and *Pasteurella multocida* bacterin SC at median ages of 2 and 70 days, respectively. The SC2 (n = 36) and SC70 (37) groups received a 7-way MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, parainfluenza 3 virus, *M haemolytica*, and *P multocida* SC at median ages of 2 and 70 days, respectively; the control group (37) remained unvaccinated until weaning. All calves received the 7-way MLV vaccine SC at median ages of 217 (weaning) and 231 days. Serum neutralizing antibody (SNA) titers against BHV1, BVDV1, and BRSV and intranasal IgA concentrations were determined at median ages of 2, 70, 140, 217, and 262 days. Cell-mediated immunity (CMI) against BHV1, BRSV, BVDV1, and *P multocida* was determined for 16 calves/group.

Results—At median ages of 140 and 217 days, BVDV1 SNA titers were significantly higher for the SC70 group than those for the other groups. Intranasal IgA concentrations and CMI increased over time for all groups. Vaccination at weaning increased SNA titers and CMI in all groups.

Conclusions and Clinical Relevance—SC administration of an MLV vaccine to 70-day-old calves significantly increased BVDV1 antibody titers before weaning. (*Am J Vet Res* 2013;74:343–354)

Effective vaccination of calves that have maternal antibodies is an important goal for cattle produc-

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From the Departments of Large Animal Medicine (Woolums, L Berghaus, K Hurley) and Population Health (R Berghaus, Ellis, Pence, Galland, D Hurley) and the Athens Veterinary Diagnostic Laboratory (Saliki), College of Veterinary Medicine, University of Georgia, Athens, GA 30602; Merck Animal Health, 8066 N 130th Rd, Cairo, NE 68824 (Burdett); and Merck Animal Health, 80 McKinley Rd, Middlebrook, VA 24459 (Nordstrom). This study was completed at the Northwest Georgia Research and Education Center, College of Agricultural and Environmental Sciences, University of Georgia, Calhoun, GA 30703.

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Address correspondence to Dr. Woolums (awoolums@uga.edu).

ABBREVIATIONS

BHV1	Bovine herpesvirus 1
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhea virus
CMI	Cell-mediated immunity
GI-FCS	Gamma-irradiated fetal calf serum
IFN	Interferon
IN	Intranasal
MLV	Modified-live virus
OD	Optical density
PBMC	Peripheral blood mononuclear cell
PI3V	Parainfluenza type 3 virus
SNA	Serum neutralizing antibody

ers because calves are susceptible to disease caused by infectious agents for which vaccines exist before maternal antibodies have completely decayed. Results of multiple studies^{1–5} indicate that vaccination of calves that have maternal antibodies can sometimes result in

an effective anamnestic immune response when those calves are subsequently exposed to a pathogen against which they were vaccinated. However, investigators of another study⁶ did not find a similar benefit for vaccination of calves that have maternal antibodies. Results of 1 study⁷ indicate that IN vaccine administration may be a more effective route of vaccination than is parenteral vaccine administration for calves that have maternal antibodies. The purpose of the study reported here was to compare the humoral and cell-mediated immune responses following administration of a multivalent MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, and PI3V at weaning between calves that received an initial, or priming, dose of the same or similar MLV vaccine via SC or IN administration while they had maternal antibodies against those pathogens. To assess the effects of age and route of priming vaccination on the immune response following the booster vaccination at weaning, calves received the priming vaccination via IN or SC administration soon after birth (median age, 2 days) or at approximately 2 months of age (median age, 70 days).

Materials and Methods

Animals—The study was approved by the University of Georgia Institutional Animal Care and Use Committee and was performed on a cow-calf operation in northwestern Georgia that was owned and operated by the University of Georgia Department of Animal and Dairy Science. The herd included purebred Angus cows and crossbred cows of predominantly Angus and Hereford breeding. Calves that were enrolled in the study were sired by either Angus or Charolais bulls, and calves born to primiparous cows were excluded from the study. Calves and their dams were housed on grass pasture and managed conventionally. No new cattle were introduced into the herd during the observation period of the study. After weaning (median calf age, 231 days), a subset 110 calves were transported to a feedlot in Iowa.

Information recorded for each calf included breed, sex, dam's parity, birth weight, weaning weight, and any medical treatments administered during the observation period. Additional information recorded for each of the calves transported to an Iowa feedlot included weight at time of transportation, average daily gain while at the feedlot, age and weight immediately prior to slaughter, and USDA yield and quality grades.

Study design—During January and February 2009, 184 calves between 1 and 5 days of age were enrolled in the study. Each calf was assigned to 1 of 5 treatment groups by systematic random allocation on the basis of birth order. Calves in the IN2 and IN70 groups were administered 2 mL of a 5-way MLV vaccine^a containing BHV1, BVDV1, BVDV2, BRSV, and PI3V IN and 2 mL of an attenuated-live *Mannheimia haemolytica* and *Pasteurella multocida* bacterin^b SC at approximately 2 or 70 days of age, respectively. Calves in the SC2 and SC70 groups were administered 2 mL of a 7-way MLV vaccine^c containing BHV1, BVDV1, BVDV2, BRSV, PI3V, *M haemolytica*, and *P multocida* SC at approximately 2 or 70 days of age, respectively. Calves in the control group were not vaccinated prior to weaning. All calves

received 2 mL of the 7-way MLV vaccine SC at weaning (median age, 217 days) and again 2 weeks later (median age, 231 days) to ensure that all calves received both a priming and booster vaccination with a multivalent vaccine against common respiratory pathogens of cattle at a time when it was likely that most calves would be immunologically responsive to vaccination and before the calves were moved to a feedlot.

Because there was a chance that calves vaccinated via the IN route might shed and transmit vaccine virus to calves in the other treatment groups during a short period immediately after vaccination, the calves in the IN2 and IN70 groups and their dams were housed separately from the rest of the herd for 14 days after administration of the priming vaccination. Also, samples from the calves in the IN2 and IN70 groups were always collected after those from the calves in the control group and the SC2 and SC70 groups to minimize the possibility of inadvertent transmission of vaccine virus by the calves in the IN2 and IN70 groups to the calves in the other treatment groups. Calves in the SC2 and SC70 groups were not separated from the calves in the control group after vaccination because the shedding and transmission of vaccine virus following SC vaccination were considered unlikely.^{8,9}

Sample collection—Because of the time required to process the various samples obtained from the study calves, the calves were allocated into 1 of 2 sampling groups such that samples were obtained from the oldest half of the calves 1 week before the youngest half of the calves at each sample collection time. From each calf within each sampling group, blood samples were collected into a glass serum separator tube^d (20 mL) and a tube containing acid citrate dextrose^d (40 to 50 mL) via jugular venipuncture when the median age of the calves in the sampling group was 2 (enrollment), 70, 140, 217, and 262 days. At the same times, nasal secretions were obtained from each calf via the following method. For each calf, a soft foam sponge^e was trimmed to an appropriate size to fit within a nostril, and a piece of cotton twine was sewn to the sponge with the free end of the twine knotted to facilitate sponge removal. The sponge was then inserted into a nostril and left in place for 2 to 3 minutes. After the sponge was removed from the nostril, it was placed into a 20- or 30-mL syringe from which the plunger had been removed. The plunger was then inserted into the syringe and used to squeeze nasal secretions from the sponge into a polypropylene tube; typically, 0.25 to 1.0 mL of nasal secretions was obtained from each sponge. Additional nasal secretions were obtained via the same method from a randomly selected subset of calves from the IN2 (n = 16), SC2 (16), and control (15) groups at a median age of 36 days to determine whether the calves had responded to the priming vaccination.

Sample processing—Within 6 hours after collection, blood and nasal secretion samples were transported on ice to the laboratory, where they were stored overnight (approx 15 hours) at 4°C. The next day, the blood samples in the serum separator tubes were centrifuged at 834 × g for 15 minutes. The serum was then removed and stored in aliquots at -20°C until analyzed for serum

total IgG concentration and SNA titers against BHV1, BVDV1, and BRSV. The nasal secretion samples were mixed with an equal volume of 0.1% Tween in PBSS, vortexed briefly, and then centrifuged at $834 \times g$ for 10 minutes. The supernatant was decanted from each sample and stored at -20°C until the sample was analyzed for total IgA concentration and BHV1-specific IgA response.

Peripheral blood mononuclear cells were isolated from all blood samples mixed with acid citrate dextrose collected at a median age of 2 days for measurement of antigen-specific production of IFN- γ , lymphocyte proliferation, or CD25 expression. For subsequent sample collections (median age, 70, 140, 217, and 262 days), PBMCs were isolated from a randomly selected subset of 16 calves from each treatment group. To isolate PBMCs, blood mixed with acid citrate dextrose was mixed with an equal volume of PBSS, layered over a mixture of polysucrose and sodium diatrizoate^f at a density of 1.077 g/mL, and centrifuged at $834 \times g$ at room temperature (22°C) for 30 minutes. The PBMCs on the surface of the polysucrose-sodium diatrizoate were removed via a pipette and transferred to a 50-mL conical tube. The PBMCs were washed twice with 40 mL of PBSS and then counted. Cell viability was confirmed via the trypan blue exclusion method. All serum, nasal secretion, and PBMC supernatant samples designated for the IFN- γ assay were stored until sample collection was completed so that all the samples could be analyzed at the same time.

Total IgG concentration in serum—For each calf, extent of passive transfer was evaluated by determination of total serum IgG concentration. Total IgG concentration was determined for serum samples collected at a median age of 2 days via an ELISA. Briefly, each well of a 96-well plate was coated with 100 μL of a 1:200 dilution of a rabbit anti-bovine IgG (heavy and light chain) polyclonal antibody^g in coating buffer (0.9% sodium carbonate in distilled water; pH, 9.7) and incubated at 4°C overnight (approx 15 hours). The plates were then washed 3 times with wash buffer (0.05% Tween in PBSS). Each serum sample was diluted with wash buffer into 3 dilutions (1:20,000, 1:100,000, and 1:500,000), as was a bovine IgG standard^h (0.5 $\mu\text{g}/\text{mL}$), which was used as a control for each plate. To each well of the 96-well plate, 100 μL of a dilution of serum or bovine IgG standard was added, and the plates were incubated at room temperature for 1 hour. Plates were washed 3 times with wash buffer, then 100 μL of a 1:1,000 dilution of peroxidase conjugated rabbit anti-bovine IgG (heavy and light chain)ⁱ in wash buffer was added to each well, and the plates were incubated at room temperature for 30 minutes. Plates were washed 4 times with wash buffer, then 50 μL of 0.001% 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt in 0.1M citric acid (pH, 4.35) containing 1% of the substrate volume of 30% H_2O_2 was added to each well. Plates were read at a light absorbance of 405 nm with a microplate reader. For each serum sample, the concentration of IgG was determined via comparison with the curve for the bovine IgG standard that was run on the same plate as that serum sample.

Total and BHV1-specific IgA concentrations in nasal secretions—Total IgA concentration in nasal secretions was determined via a commercially available ELISA kit^j for quantification of bovine IgA. The detection antibody for the kit was used at a dilution of 1:50,000. The total IgA concentration in each nasal secretion sample was determined via comparison with the curve for the kit standard that was run on the same plate as that sample.

To determine BHV1-specific IgA response in nasal secretions, each well of a 96-well plate was coated with 100 μL of a 1:400 dilution of binary ethyleneimine-inactivated BHV1 Cooper strain^k in coating buffer and incubated at 4°C for 12 to 18 hours. This dilution was determined to yield maximal BHV1-specific signal from the serum of 2 vaccinated calves and minimal background signal from the serum of the same 2 calves prior to vaccination, and also from fetal bovine serum. Following incubation, the plates were washed 3 times with wash buffer. The plates were prepared in large batches, blotted dry, and stored at -20°C until used. On the day that the assay was performed, the required number of plates was removed from the freezer. Each plate was washed once with wash buffer. To each well, 200 μL of PBSS solution containing 0.5% bovine serum albumin^l was added, and the plates were incubated at room temperature for 1 hour. The plates were washed 3 times with wash buffer. Nasal secretion samples, which had been stored frozen at a 1:2 dilution in 0.1% Tween and PBSS, were thawed and further diluted 1:5 with wash buffer, resulting in a final sample dilution of 1:10. For each nasal secretion sample, 100 μL of the 1:10 diluted sample was added to each of 4 wells of the 96-well plate. The plates were incubated at room temperature for 1 hour and then washed 4 times with wash buffer. To each well, a 1:50,000 dilution of horseradish peroxidase-conjugated sheep anti-bovine IgA^m was added, and the plates were incubated at room temperature for 1 hour. The plates were washed 4 times with wash buffer, and 100 μL of 0.001% 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt in 0.1M citric acid (pH, 4.35) containing 1% of the substrate volume of 30% H_2O_2 was added to each well. The plates were incubated at room temperature for 20 to 40 minutes or until the expected color intensity was observed for the positive control samples. Plates were read at a light absorbance of 405 nm with a microplate reader. Each well was qualitatively scored on a 0 to 3 scale: 0 = OD of well was \leq twice the OD of the negative control (fetal bovine serum) well, 1 = OD of well was $>$ twice the OD of the negative control well but \leq twice the OD of the positive control well, 2 = OD of well was $>$ 2 but \leq 4 times the OD of the positive control well, and 3 = OD of well was $>$ 4 times the OD of the positive control well.

SNA titers against BHV1, BVDV1, and BRSV—Serum neutralizing antibody titers against BHV1, BVDV1, and BRSV were determined via a standard virus neutralization test.¹⁰ Briefly, serum samples were thawed and heat inactivated at 56°C for 30 minutes. Heat-inactivated serum samples were then diluted with Dulbecco minimum essential medium into serial 2-fold dilutions, and 25 μL of each dilution was added to each of 2 wells

on a 96-well plate such that there were duplicate columns of serial dilutions for each sample. To each well, an equal volume (25 μL) of Dulbecco minimum essential medium that contained 100 TCID₅₀ of the appropriate virus was added. The plates were incubated in 5% CO₂ at 37°C for 1 hour. Then 150 μL (approx 10⁴ cells) of a Madin-Darby bovine kidney cell suspension was added to each well. The plates were incubated in 5% CO₂ at 37°C for 4 days. After incubation, an inverted microscope was used to evaluate the cell monolayer in each well for virus-specific cytopathic effects. The SNA titer for each sample was reported as the highest dilution of serum that completely prevented virus-induced cytopathic effects in both wells.

IFN- γ secretion by PBMCs in response to stimulation with BHV1, BVDV1, BRSV, or *P multocida*—After PBMCs were isolated and counted, the concentration of PBMCs in each sample was titrated to 10⁷ PBMCs/mL with a mixture of RPMI medium, 10% GI-FCS, and 1% penicillin-streptomycin, and then 100- μL aliquots were transferred to replicates of 3 wells in a 96-well flat-bottom plate. To each well of 1 replicate of 3 wells, 100 μL of binary ethyleneimine-inactivated BHV1 Cooper strain^k (10⁷ TCID₅₀/mL), live BVDV NADL strain (10^{6.2} TCID₅₀/mL), live BRSV strain 375 (5.6 \times 10⁶ TCID₅₀/mL), inactivated *P multocida*^k (2.9 \times 10¹⁰ CFUs/mL), concanavalin A (10 $\mu\text{g}/\text{mL}$), or a mixture of RPMI medium, 10% GI-FCS, and 1% penicillin-streptomycin (cell culture media) was added. Plates were then incubated at 37°C in 5% CO₂ for 120 hours. After incubation, the contents of wells were transferred to wells of 96-well round-bottom plates and centrifuged at 200 \times g for 5 minutes. From each well, 150 μL of supernatant was transferred to another 96-well flat-bottom plate and stored at -80°C until analyzed. Secretion of IFN- γ by PBMCs in response to stimulation with BHV1, BVDV1, BRSV, and *P multocida* was measured via a commercially available ELISAⁿ in accordance with the manufacturer's instructions. For each sample, the IFN- γ concentration was determined via comparison with the curve generated by the IFN- γ standard on the same plate as that sample, and results were reported in picograms per milliliter. The concentration of IFN- γ secreted by PBMCs in response to each antigen was calculated by subtraction of the IFN- γ concentration determined for PBMCs cultured in cell culture media only from the IFN- γ concentration determined for PBMCs cultured with BHV1, BVDV1, BRSV, *P multocida*, or concanavalin A, respectively.

PBMC proliferation in response to stimulation with BHV1, BVDV1, BRSV, or *P multocida*—After PBMCs were isolated and counted, PBMCs were suspended in RPMI medium, 10% GI-FCS, and 1% penicillin-streptomycin to yield a concentration of 5 \times 10⁶ cells/mL. For each sample, 50 μL of the PBMC suspension was added to replicates of 4 wells of a 96-well flat-bottom plate. To each well of 1 replicate of 4 wells, 50 μL of binary ethyleneimine-inactivated BHV1 Cooper strain (10⁷ TCID₅₀/mL), live BVDV NADL strain (10^{6.2} TCID₅₀/mL), live BRSV strain 375 (5.6 \times 10⁶ TCID₅₀/mL), inactivated *P multocida* (2.9 \times 10¹⁰ CFUs/mL), concanavalin A (10 $\mu\text{g}/\text{mL}$), *Staphylococcus enterotoxin B* (0.2 $\mu\text{g}/\text{mL}$) or a mixture of RPMI medium,

10% GI-FCS, and 1% penicillin-streptomycin (cell culture media) was added. The plates were incubated in 5% CO₂ at 37°C for 120 hours. After incubation, 10 μL of ³H-thymidine (0.2 μCi) was added to each well, and the plates were incubated in 5% CO₂ at 37°C for an additional 6 hours. Well contents were harvested onto glass fiber filters and read on a scintillation counter. Peripheral blood mononuclear cell proliferation in response to each antigen or mitogen was determined via subtraction of the mean PBMC count per minute for PBMCs cultured in cell culture media only from the mean PBMC count per minute for PBMCs cultured with BHV1, BVDV1, BRSV, *P multocida*, or mitogens, respectively.

CD25 expression by total PBMCs and the CD4, CD8, and $\gamma\Delta$ T-cell subsets of PBMCs in response to stimulation with BHV1, BVDV1, BRSV, or *P multocida*—After PBMCs were isolated and counted, the concentration of PBMCs in each sample was titrated to 10⁷ PBMCs/mL with a mixture of RPMI medium, 10% GI-FCS, and 1% penicillin-streptomycin, and then 100- μL aliquots were transferred to replicates of 4 wells in a 96-well flat-bottom plate. To each well of 1 replicate of 4 wells, 100 μL of binary ethyleneimine-inactivated BHV1 Cooper strain (10⁷ TCID₅₀/mL), live BVDV NADL strain (10^{6.2} TCID₅₀/mL), live BRSV strain 375 (5.6 \times 10⁶ TCID₅₀/mL), inactivated *P multocida* (2.9 \times 10¹⁰ CFUs/mL), concanavalin A (10 $\mu\text{g}/\text{mL}$), *Staphylococcus enterotoxin B* (0.2 $\mu\text{g}/\text{mL}$) or a mixture of RPMI medium, 10% GI-FCS, and 1% penicillin-streptomycin (cell culture media) was added. Following incubation,

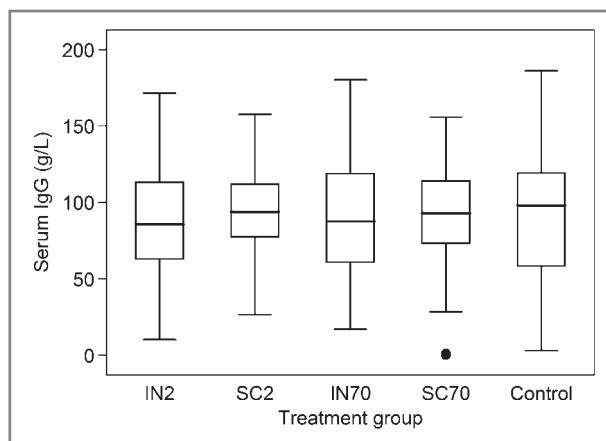


Figure 1—Box-and-whiskers plot of serum total IgG concentration by treatment group for 179 beef crossbred calves at study enrollment (median calf age, 2 days). Calves were systematically allocated to 1 of 5 treatment groups via birth order. Calves in the IN2 (n = 37) and IN70 (36) groups were administered 2 mL of a 5-way MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, and PI3V IN and 2 mL of an attenuated live *Mannheimia haemolytica* and *Pasteurella multocida* bacterin SC at a median age of 2 or 70 days, respectively. Calves in the SC2 (n = 35) and SC70 (37) groups were administered 2 mL of a 7-way MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, PI3V, *M haemolytica*, and *P multocida* SC at a median age of 2 or 70 days, respectively. Calves in the control group (n = 34) were not vaccinated prior to weaning. All calves received 2 mL of the 7-way MLV vaccine SC at weaning (median age, 217 days) and again 2 weeks later (median age, 231 days). Each box represents the interquartile range (25th to 75th percentiles), the horizontal line in each box represents the median, and the vertical lines and brackets (whiskers) represent the range. The black dot in the SC70 column represents an outlier value.

the contents of each well were transferred to the corresponding well of a 96-well round-bottom plate, and the round-bottom plate was centrifuged at 500 × g at 4°C for 5 minutes. The supernatant was discarded, and the plates were washed twice (200 µL/well) with flow cytometry buffer (PBSS with 2% bovine serum albumin and 1% sodium azide). The cells were then labeled with a murine monoclonal antibody against bovine CD25 conjugated to R-phycoerythrin^o only or with a murine monoclonal antibody against bovine CD25 conjugated to R-phycoerythrin in combination with a murine monoclonal antibody against bovine CD4 conjugated to fluorescein isothiocyanate,^p a murine monoclonal antibody against bovine CD8 conjugated to fluorescein isothiocyanate,^q or a murine monoclonal antibody against WC1 (to identify γΔ T cells) conjugated to fluorescein isothiocyanate.^r To each well, 20 µL of the appropriate antibody solution diluted 1:10 with flow cytometry buffer was added, and the plates were incubated at 4°C for 20 minutes. The plates were washed twice (200 µL/well) with flow cytometry buffer. Cells within each well were then fixed with flow cytometry fixation buffer (200 mL of 10% formalin solution [made with PBSS] added to 300 mL of flow cytometry buffer). The percentage of total PBMCs expressing CD25 in response to each stimulant was evaluated via flow cytometry.^s Additionally, expression of CD25 by the large (ie, activated) subpopulation of PBMCs in response to each stimulant was also measured via flow cytometry. For cells dual stained for CD25 and CD4, CD8, or the γΔ T-cell receptors, the percentage of double-positive cells multiplied by the mean fluorescence intensity of double-positive cells was determined for each stimulant.

Statistical analyses— χ^2 analyses were used to compare the distribution of breed and sex among the treatment groups. A Kruskal-Wallis test was used to compare dam parity among the treatment groups. Body weight and serum total IgG concentration of calves at enrollment were compared among the treatment groups via 1-way ANOVA. Bovine herpes virus 1-specific IgA response was recorded on an ordinal scale; therefore, for each sample collection time, the BHV1-specific IgA response was compared among the treatment groups via a Kruskal-Wallis test. Changes in BHV1-specific IgA response over time were compared among the treatment groups via a Skillings-Mack test. Repeated-measures ANOVA was used to compare total IgA concentration, logarithmically transformed SNA titers, IFN- γ concentration, PBMC proliferation, and percentage of PBMCs with various cell markers among the treatment groups over time. For within-subject variables, the *F* test degrees of freedom were adjusted via the Greenhouse-Geisser estimate of epsilon to correct for departures from the sphericity assumption. Post hoc tests were performed via the Bonferroni procedure to limit the type I error rate to 5% for all comparisons.

For the subset of calves that were sent to an Iowa feedlot, a 1-way ANOVA was used to compare age at slaughter, weight at slaughter, and average daily gain while at the feedlot among the treatment groups. Exact tests of independence were used to compare the proportion of calves treated with antimicrobials and the carcass quality grade among treatment groups, and a

Kruskal-Wallis test was used to compare USDA yield grade among treatment groups.

All analyses were performed with commercially available statistical software.¹ The distribution of continuous variables was evaluated for normality via examination of histograms and box plots. For all analyses, the alternative hypothesis was assumed to be 2 sided, and values of *P* < 0.05 were considered significant.

Results

Animals—Of the 184 calves enrolled in the present study, 5 (3 from the control group, 1 from the SC2 group, and 1 from the IN70 group) were removed from the study prior to its completion. The reasons these calves were removed from the study included death shortly after birth as a result of a congenital heart defect (*n* = 1), leg injuries that resulted in euthanasia (2), inadvertent sample collection from a calf < 12 hours old

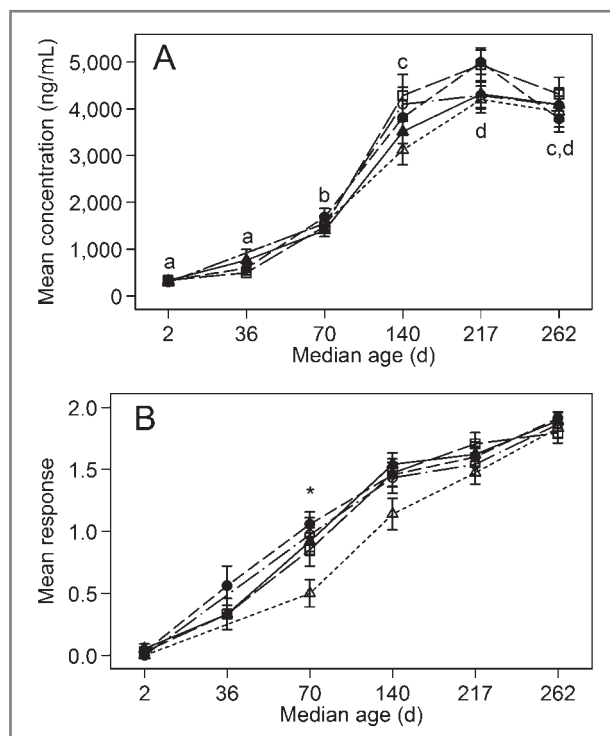


Figure 2—Mean \pm SEM total IgA concentration (A) and BHV1-specific IgA response (B) in nasal secretions obtained from calves in the IN2 (black triangles with solid line; *n* = 37), IN70 (white triangles with dashed line; 37), SC2 (black circles with dashed line; 35), SC70 (white circles with dashed line; 37), and control (white squares with dashed line; 34) groups at a median age of 2, 36, 70, 140, 217, and 262 days. At the median age of 36 days, nasal secretions were obtained from only 16 calves in each of the IN2 and SC2 groups and 15 calves in the control group. The BHV1-specific IgA response in each sample was qualitatively scored on a 0 to 3 scale: 0 = OD of sample was \leq twice the OD of the negative control, 1 = OD of sample was > twice the OD of the negative control but \leq twice the OD of the positive control, 2 = OD of sample was > 2 but \leq 4 times the OD of the positive control, and 3 = OD of sample was > 4 times the OD of the positive control. Mean total IgA concentration did not vary significantly among the treatment groups at any sample collection time. ^{a-d}Within a treatment group, values with different superscript letters differ significantly (*P* < 0.05). *Value for the IN70 group is significantly (*P* = 0.011) less than that for the SC2 group. See Figure 1 for remainder of key.

(1), and death of the calf's dam (1). As a result, data were obtained and analyzed for only 179 calves (IN2 group, $n = 37$; SC2 group, 35; IN70 group, 36; SC70 group, 37; control group, 34).

Of the 179 calves included in the analyses, 79 (44.1%) were Angus or Angus-Hereford crossbred, 100 (55.9%) were Charolais crossbred, 96 (53.6%) were male, and 83 (46.4%) were female. The distribution of breed ($P = 0.95$) and sex ($P = 0.50$) did not vary significantly among treatment groups. The dam parity for the study calves ranged from 2 to 13 (median, 5) and did not vary significantly ($P = 0.18$) among the treatment

groups. The mean \pm SEM weight of calves at birth (38.7 ± 0.4 kg; $P = 0.43$), weaning (285.8 ± 2.6 kg; $P = 0.31$), and transportation to an Iowa feedlot (320.3 ± 2.6 kg; $P = 0.41$) did not vary significantly among the treatment groups. Between birth and transportation to the feedlot, none of the study calves were treated for respiratory disease; however, 10 calves were treated for various other conditions, including foot rot ($n = 5$), foot injuries (2), diarrhea (2), and ear infection (1).

The 110 calves transported to an Iowa feedlot included 23 from the IN2 group, 21 from the SC2 group, 20 from the IN70 group, 23 from the SC70 group, and 23 from the control group. Of these 110 calves, 47 (42.7%) were heifers and 63 (57.3%) were steers, and 109 (99.0%) calves completed the feeding period (1 calf in the IN2 group died prior to processing at the feedlot). For the 109 calves that completed the feeding period, age at slaughter ($P = 0.82$), weight at slaughter ($P = 0.86$), average daily gain while at the feedlot ($P = 0.98$), percentage of calves treated with antimicrobials ($P = 0.85$), USDA yield grade ($P = 0.82$), and USDA quality grade ($P = 0.32$) did not vary significantly among the treatment groups. The number of antimicrobial treatments administered to calves during the feeding period ranged from 1 to 3/group.

Total IgG concentration in serum—Mean \pm SEM serum total IgG concentrations by group were as follows: IN2, 90.9 ± 7.1 g/L; SC2, 94.6 ± 5.1 g/L; IN70, 93.1 ± 6.5 g/L; SC70, 89.9 ± 6.1 g/L; and control, 91.9 ± 7.4 g/L. Median serum total IgG concentration for calves at study enrollment (median age, 2 days) did not vary significantly ($P = 0.99$) among the treatment groups (Figure 1). The use of a serum IgG concentration of ≥ 16 g/L as the cutoff for adequate passive transfer¹¹ resulted in 4 (2.2%) calves being classified with inadequate passive transfer.

Total and BHV1-specific IgA concentrations in nasal secretions—Total IgA concentration in nasal secretions increased significantly ($P < 0.001$) during the observation period for all treatment groups; however, treatment group ($P = 0.31$) and the interaction between treatment group and sample collection time ($P = 0.43$) were not associated with total IgA concentration in nasal secretions (Figure 2). Similarly, BHV1-specific IgA response in nasal secretions increased significantly ($P < 0.001$) during the observation period for all treatment groups. Among the treatment groups, BHV1-specific IgA response did not vary significantly for nasal secretions obtained from calves at a median age of 2 ($P = 0.48$), 36 ($P = 0.48$), 140 ($P = 0.16$), 217 ($P = 0.35$), or 262 ($P = 0.73$) days. The BHV1-specific IgA response in nasal secretions did vary significantly ($P = 0.011$) for samples obtained from calves at a median age of 70 days; the mean BHV1-specific IgA response in nasal secretions was significantly higher for the SC2 group, compared with that for the IN70 group, although the absolute difference was not large.

BHV1, BVDV1, and BRSV SNA titers—The geometric mean SNA titers against BHV1 and BRSV decreased significantly ($P < 0.001$) in a similar manner during the observation period for all treatment groups.

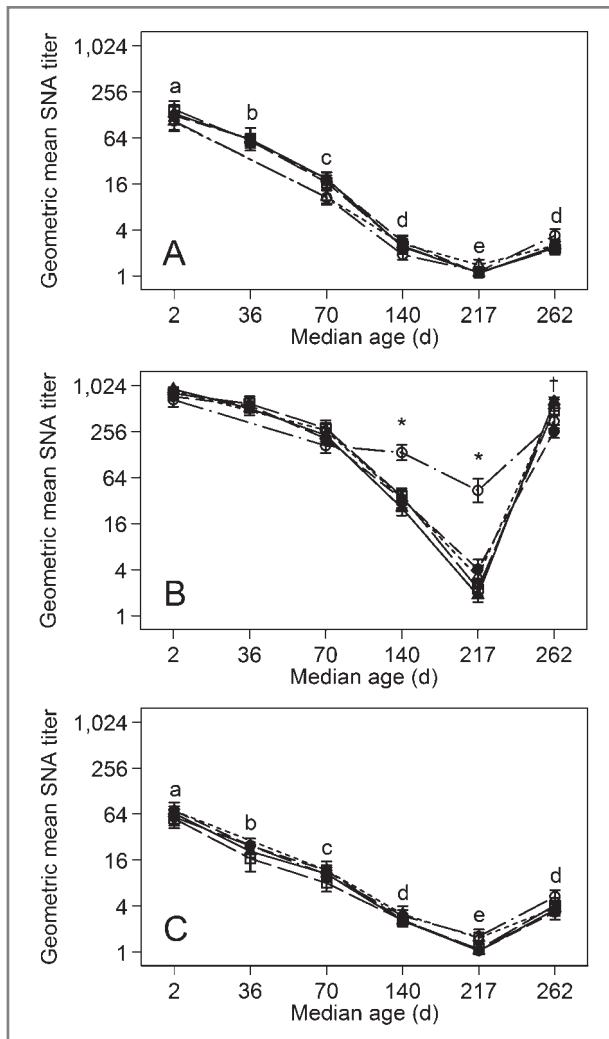


Figure 3—Geometric mean \pm SEM SNA titers against BHV1 (A), BVDV1 (B), and BRSV (C) for calves in the IN2 (black triangles with solid line; $n = 37$), IN70 (white triangles with dashed line; 37), SC2 (black circles with dashed line; 35), SC70 (white circles with dashed line; 37), and control (white squares with dashed line; 34) groups at a median age of 2, 36, 70, 140, 217, and 262 days. Serum neutralizing antibody titer data were logarithmically transformed (\log_2) to normalize the distribution of the data. Geometric mean SNA titers against BHV1 and BRSV did not vary significantly among the treatment groups at any sample collection time. ^{a-e}Within a treatment group, values with different superscript letters differ significantly ($P < 0.05$). *Value for SC70 group differs significantly ($P < 0.05$) from the values for the other 4 treatment groups. †Value for the SC2 group differs significantly ($P < 0.05$) from the values for the IN2 and IN70 groups. See Figure 1 for remainder of key.

Treatment group and the interaction between treatment group and sample collection time were not associated with BHV1 or BRSV SNA titers (Figure 3).

The geometric mean BVDV1 SNA titer decreased over time for all treatment groups until the calves were weaned at a median age of 217 days and were administered the 7-way MLV vaccine, after which the geometric mean BVDV1 SNA titer increased for all treatment groups (Figure 3). The interaction between treatment group and sample collection time was significantly ($P < 0.001$) associated with BVDV SNA titer. The SC70 group had a significantly higher BVDV1 SNA titer, compared with those of the other 4 treatment groups, when calves were a median age of 140 and 217 days. At a median age of 262 days, calves in the SC2 group had a significantly lower mean BVDV1 SNA titer than did calves in the IN2 and IN70 groups, whereas the mean BVDV1 SNA titer for calves in the control group did not vary significantly from those of the other 4 treatment groups. There was a > 4-fold increase in BVDV1 SNA titers for all calves between a median age of 217 and 262 days.

IFN- γ secretion by PBMCs and PBMC proliferation in response to stimulation with BHV1, BVDV1, BRSV, or *P. multocida*—The concentration of IFN- γ secreted by PBMCs in response to stimulation with

BHV1, BVDV1, BRSV, or *P. multocida* for a subset of 16 calves in each treatment group during the observation period was plotted (Figure 4). Treatment group and the interaction between treatment group and sample collection time were not associated with IFN- γ secretion by PBMCs in response to stimulation with any of the stimulants evaluated. Secretion of IFN- γ by PBMCs stimulated with BHV1 was significantly greater at a median age of 262 days, compared with that at a median age of 217 days, for all treatment groups.

For the same subset of calves in each treatment group, proliferation of PBMCs in response to stimulation with BHV1, BVDV1, BRSV, or *P. multocida* during the observation period was plotted (Figure 5). Treatment group and the interaction between treatment group and sample collection time were not associated with PBMC proliferation in response to stimulation with any of the stimulants evaluated. Proliferation by PBMCs after stimulation with BHV1, BVDV1, and *P. multocida* was significantly greater at a median age of 262 days, compared with that at a median age of 217 days.

For PBMCs exposed to BHV1 and BVDV1, the pattern of CMI responsiveness as measured via IFN- γ secretion differed from the pattern of CMI responsiveness as measured via proliferation. For PBMCs exposed to BHV1, the highest mean IFN- γ concentrations were

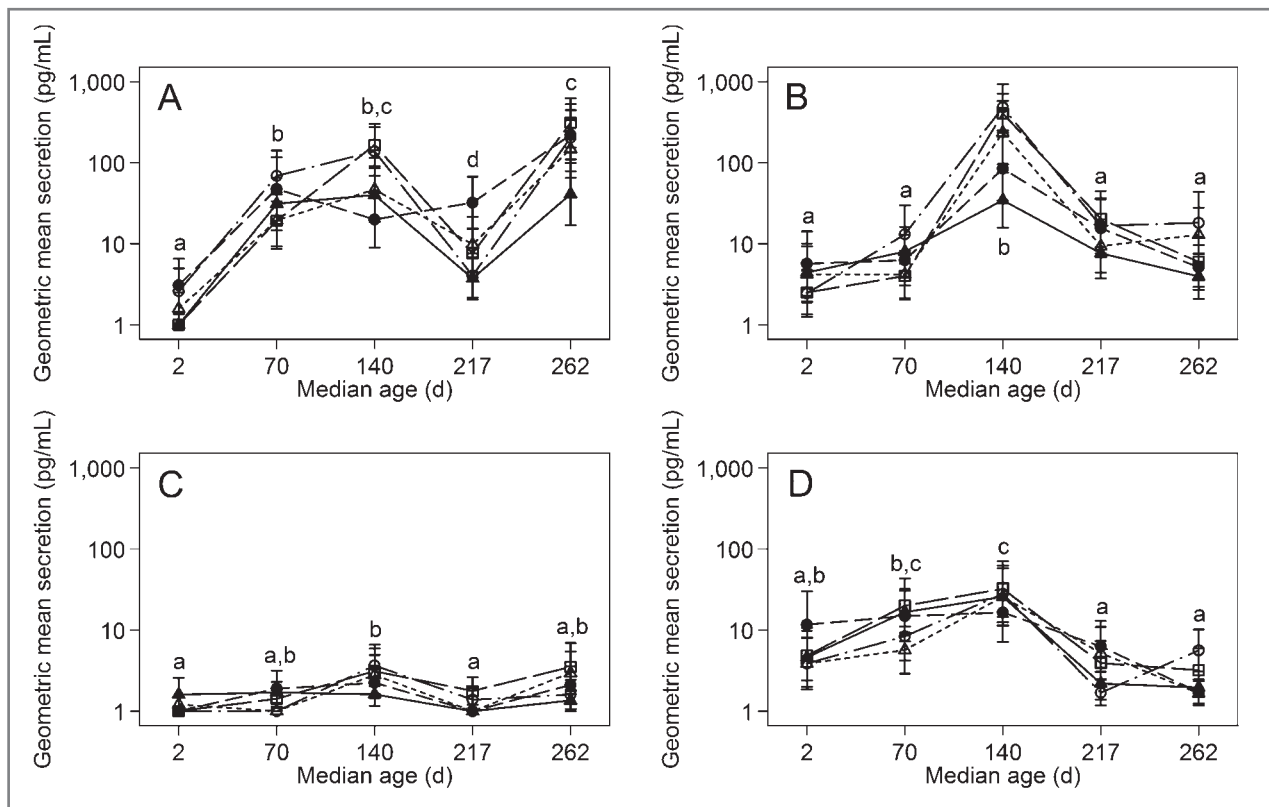


Figure 4—Geometric mean \pm SEM secretion of IFN- γ by PBMCs in response to stimulation with BHV1 (A), BVDV1 (B), BRSV (C), and *Pasteurella multocida* (D) for calves in the IN2 (black triangles with solid line; $n = 16$), IN70 (white triangles with dashed line; 16), SC2 (black circles with dashed line; 16), SC70 (white circles with dashed line; 16), and control (white squares with dashed line; 15) groups at a median age of 2, 70, 140, 217, and 262 days. At a median age of 2 days, values represent data from only 12 calves from each of the IN2 and SC2 groups, 13 calves from each of the IN70 and control groups, and 14 calves from the SC70 group. Interferon- γ secretion data were logarithmically transformed (\log_{10}) to normalize the distribution of the data. Geometric mean secretion of IFN- γ by PBMCs did not vary significantly among the treatment groups at any sample collection time for any of the pathogens evaluated. ^{a-d}Within a treatment group, values with different superscript letters differ significantly ($P < 0.05$). See Figure 1 for remainder of key.

detected in samples that were obtained from calves at a median age of 140 and 262 days, whereas the highest mean values for PBMC proliferation were detected in samples that were obtained from calves at a median age of 2 and 262 days, and the lowest mean value for PBMC proliferation was detected in samples that were obtained from calves at a median age of 140 days. Similarly, for PBMCs exposed to BVDV1, the highest mean IFN- γ concentration was detected in samples obtained from calves at a median age of 140 days, whereas the highest mean value for PBMC proliferation was detected in samples obtained from calves at a median age of 2 days, and the lowest mean value for PBMC proliferation was detected in samples obtained from calves at a median age of 140 days.

CD25 expression by total PBMCs and the CD4, CD8, and $\gamma\Delta$ T-cell subsets of PBMCs in response to stimulation with BHV1, BVDV1, BRSV, or *P multocida*—The mean percentage of total PBMCs that expressed CD25 following stimulation with BHV1, BVDV1, BRSV, or *P multocida* varied significantly ($P < 0.001$) during the observation period (Table 1). Treatment group and the interaction between treatment group and sample collection time were not associated with CD25 expression by PBMCs; therefore, the mean percentages of total PBMCs that expressed CD25 for all treatment groups

combined were reported. For PBMCs that were exposed to cell culture media only (ie, background response), the mean percentage of PBMCs that expressed CD25 also varied during the observation period; however, the background response for PBMCs obtained from calves at a median age of 2 days did not differ significantly from the background response for PBMCs obtained from calves at a median age of 262 days. The percentage of PBMCs that expressed CD25 after stimulation with BHV1, BVDV1, or BRSV for PBMCs obtained at a median age of 262 days was significantly greater, compared with that for PBMCs obtained at a median age of 2 days. Also, the percentage of PBMCs that expressed CD25 after stimulation with BHV1 or BRSV for PBMCs obtained at a median age of 262 days was significantly greater, compared with that for PBMCs obtained at a median age of 217 days.

Similarly, the percentage of large (ie, activated) PBMCs that expressed CD25 after stimulation with BHV1, BVDV1, BRSV, or *P multocida* varied significantly during the observation period for all treatment groups. Treatment group and the interaction between treatment group and sample collection time were not associated with CD25 expression by large PBMCs. For all stimulants, the percentage of large PBMCs that expressed CD25 was significantly greater for PBMCs that were obtained from calves at a median age of 262 days, compared

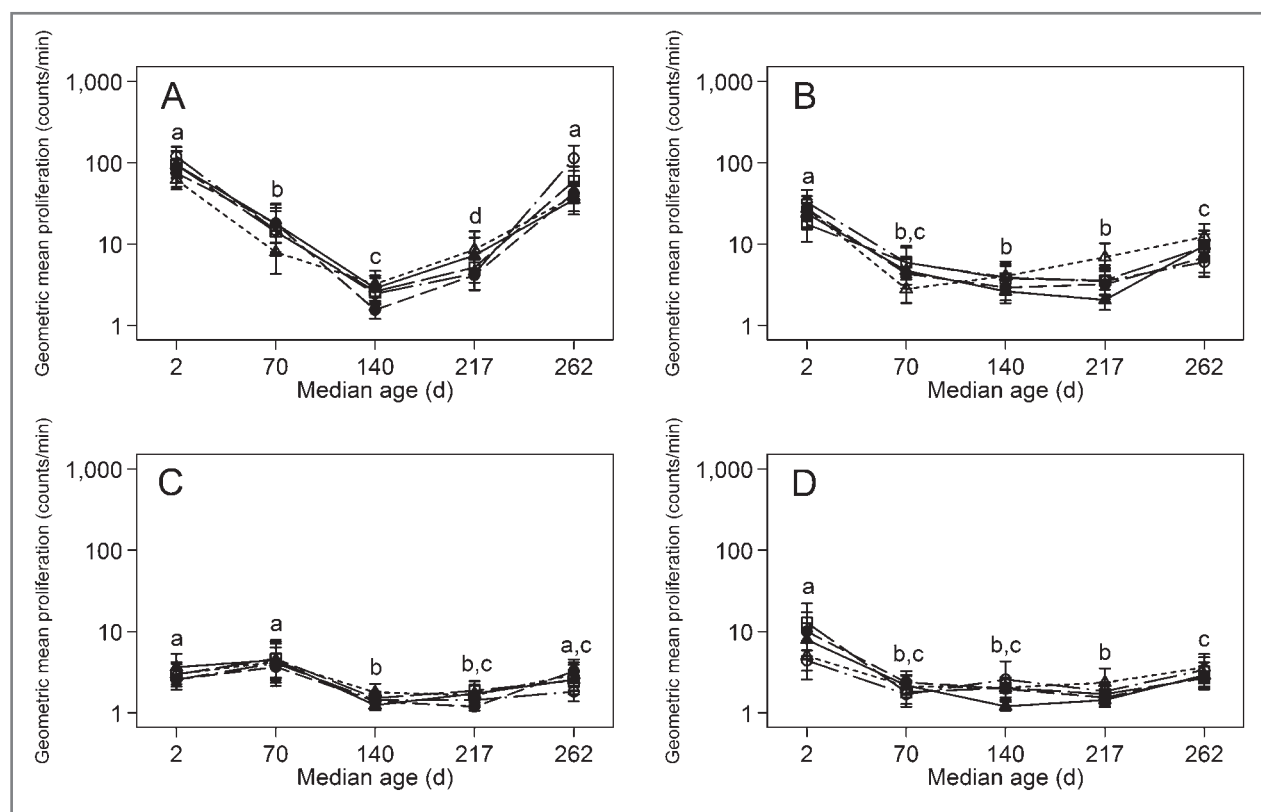


Figure 5—Geometric mean \pm SEM PBMC proliferation in response to stimulation with BHV1 (A), BVDV1 (B), BRSV (C), and *P multocida* (D) for calves in the IN2 (black triangles with solid line; $n = 16$), IN70 (white triangles with dashed line; 16), SC2 (black circles with dashed line; 16), SC70 (white circles with dashed line; 16), and control (white squares with dashed line; 15) groups at a median age of 2, 70, 140, 217, and 262 days. At a median age of 2 days, values represent data from only 12 calves from each of the IN2 and SC2 groups, 13 calves from each of the IN70 and control groups, and 14 calves from the SC70 group. Peripheral blood mononuclear cell proliferation data were logarithmically transformed (\log_{10}) to normalize the distribution of the data. Geometric mean PBMC proliferation did not vary significantly among the treatment groups at any sample collection time for any of the pathogens evaluated. ^{a-d}Within a treatment group, values with different superscript letters differ significantly ($P < 0.05$). See Figure 1 for remainder of key.

Table 1—Mean \pm SEM percentage CD25 expression by total PBMCs in response to stimulation with cell culture medium alone, BHV1, BVDV1, BRSV, or *Pasteurella multocida* in 179 beef crossbred calves* at a median age of 2, 70, 140, 217, and 262 days.

Stimulant	Median age (d)				
	2	70	140	217	262
Cell culture medium	10.6 \pm 1.0 ^{a,b}	10.1 \pm 0.7 ^a	16.1 \pm 1.2 ^c	13.1 \pm 0.9 ^{b,c}	11.7 \pm 0.7 ^{a,b}
BHV1	14.0 \pm 1.1 ^a	14.1 \pm 0.8 ^a	22.0 \pm 1.4 ^b	18.3 \pm 0.9 ^c	23.7 \pm 0.5 ^b
BVDV	15.4 \pm 1.2 ^{a,b}	15.0 \pm 0.9 ^a	25.1 \pm 1.4 ^c	19.4 \pm 0.9 ^{b,d}	21.5 \pm 0.9 ^{c,d}
BRSV	14.5 \pm 1.1 ^{a,b}	12.3 \pm 0.8 ^a	19.9 \pm 1.3 ^c	17.0 \pm 0.9 ^b	21.4 \pm 0.8 ^c
<i>P multocida</i>	30.6 \pm 1.9 ^a	20.7 \pm 1.0 ^b	30.2 \pm 1.6 ^a	27.0 \pm 0.8 ^a	26.4 \pm 0.9 ^a

*Calves were systematically allocated to 1 of 5 treatment groups via birth order. Calves in the IN2 (n = 37) and IN70 (36) groups were administered 2 mL of a 5-way MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, and PI3V IN and 2 mL of an attenuated live *Mannheimia haemolytica* and *P multocida* bacterin SC at a median age of 2 or 70 days, respectively. Calves in the SC2 (n = 35) and SC70 (37) groups were administered 2 mL of a 7-way MLV vaccine containing BHV1, BVDV1, BVDV2, BRSV, PI3V, *M haemolytica*, and *P multocida* SC at a median age of 2 or 70 days, respectively. Calves in the control group (n = 34) were not vaccinated prior to weaning. All calves received 2 mL of the 7-way MLV vaccine SC at weaning (median age, 217 days) and again 2 weeks later (median age, 231 days). Because the mean percentage CD25 expression by PBMCs did not vary among the treatment groups at any sample collection time for any of the stimulants evaluated, the mean percentage CD25 expression by PBMCs for all treatment groups combined is reported.

^{a-d}Within a stimulant, values with different superscript letters differ significantly ($P < 0.05$).

with that for PBMCs that were obtained from calves at a median age of 2 days. Conversely, for all stimulants, the percentage of large PBMCs that expressed CD25 did not differ significantly for PBMCs that were obtained from calves at a median age of 262 days, compared with that for PBMCs that were obtained from calves at a median age of 217 days. The percentage of large PBMCs that expressed CD25 was greater than the percentage of total PBMCs that expressed CD25 following exposure to any evaluated stimulant. The mean percentage of total PBMCs that expressed CD25 after exposure to the evaluated stimulants ranged from 12% to 31%, whereas the mean percentage of large PBMCs that expressed CD25 after exposure to the evaluated stimulants ranged from 19% to 69%. Generally, the mean percentage of total or large PBMCs that expressed CD25 was greater after stimulation with *P multocida*, compared with that after stimulation with BHV1, BVDV1, or BRSV (Table 1).

The percentage of CD4, CD8, or $\gamma\Delta$ T cells that expressed CD25 following stimulation with BHV1 or BVDV increased during the observation period; however, treatment group and the interaction between treatment group and sample collection time were not associated with CD25 expression by CD4, CD8, or $\gamma\Delta$ T cells. The percentage of CD4, CD8, or $\gamma\Delta$ T cells that expressed CD25 following stimulation with *P multocida* did not increase during the observation period. Generally, CD25 expression by CD4, CD8, and $\gamma\Delta$ T cells after stimulation with BHV1, BVDV1, or BRSV followed a similar pattern. The percentage of CD4, CD8, or $\gamma\Delta$ T cells that expressed CD25 after stimulation with BHV1, BVDV1, or BRSV was significantly greater for cells obtained from calves at a median age of 262 days, compared with that for cells obtained from calves at a median age of 217 days.

Discussion

Results of the present study suggested that the anamnestic humoral and CMI responses in beef calves following administration of a 7-way MLV booster vaccination containing BHV1, BVDV1, BVDV2, BRSV, PI3V,

M haemolytica, and *P multocida* SC at weaning were not affected by the age at which calves received the initial, or priming, vaccination or by the route (IN vs SC) by which that priming vaccination was administered. In the present study, calves were vaccinated at a median age of 2 or 70 days, ages at which calves were expected to have high concentrations of maternal antibodies. Historically, vaccination of calves that have maternal antibodies has been considered problematic because of vaccine inactivation by the maternal antibodies¹; however, results of multiple studies^{3-5,12-18} indicate that calves may benefit from administration of a priming dose of a vaccine while they still have maternal antibodies. Parenteral (SC or IM) administration of a multivalent MLV or inactivated (killed) vaccine to calves that have maternal antibodies slowed the decay of SNA titers against BHV1, BVDV, and PI3V^{3,12-14} and induced an anamnestic response when the calves were subsequently administered a booster vaccination^{15,16} or challenge exposed to a pathogen against which they had been vaccinated.³⁻⁵ In the present study, calves in the SC70 group had significantly higher titers of BVDV1 antibodies after the initial vaccination and before the booster vaccination at weaning, compared with those for calves in the other 4 treatment groups. In fact, the BVDV1 SNA titers for calves in the SC70 group between a median age of 70 and 217 days were similar to those associated with protection against disease following challenge exposure with BVDV for calves that were vaccinated at 4 months of age¹⁷ and fetal protection against BVDV infection for pregnant cows that were exposed to cattle persistently infected with BVDV.¹⁸ Thus, SC administration of a priming dose of a vaccine that contains BVDV antigens to 70-day-old calves that have maternal antibodies may provide protection against disease should those calves be subsequently exposed to BVDV.

In the present study, the mean serum total IgG concentration determined for calves at study enrollment (median age, 2 days) did not vary significantly among the 5 treatment groups. Thus, it appeared that calves within each treatment group had a similar immunologic status at study enrollment, and it was unlikely

that any subsequent variations in mean measures of immune system function among the treatment groups were caused by differences in the initial immunologic status of the calves within those groups. The mean serum total IgG concentration at enrollment for each of the 5 treatment groups of the present study was higher than the mean serum total IgG concentration (30.4 g/L) reported for 601 beef calves (age, 2 to 8 days) in western Canada¹⁹ and the mean serum total IgG1 concentration (18.9 g/L) reported for 225 beef calves in eastern Canada.²⁰ However, in the present study, the serum total IgG concentration was measured via an ELISA, whereas it was measured via radial immunodiffusion in the other studies^{19,20} so the values may not be directly comparable among the 3 studies.

The increase in the mean BHV1-specific IgA response in nasal secretions among all 5 treatment groups during the observation period of the present study was unexpected, and its clinical relevance is unknown. Given that the total IgA concentration in nasal secretions also increased during the observation period, the increased BHV1-specific IgA response in nasal secretions may have been the result of a bystander effect from nonspecific stimulation of the mucosal immune response as the calves aged. Alternatively, the increase in BHV1-specific IgA responses during the observation period may have been caused by natural exposure of the calves to BHV1. Prior to initiation of the present study, we recognized that the use of conventionally managed calves was accompanied with a risk that the study calves could be naturally exposed to any of the pathogens evaluated. However, a benefit of the use of calves from a single large cow-calf operation was that a large number of calves could be included in each treatment group, and this benefit was considered to outweigh the risk of calves becoming naturally exposed to pathogens. Although we cannot rule out the possibility of subclinical infection with BHV1 in the calves of the present study, the fact that none of the cattle in the herd developed clinical signs of BHV1 infection and no new cattle were introduced into the herd during the study suggests that natural exposure to BHV1 was unlikely.

In the present study, considerable effort was made to prevent transmission of vaccine strains of viruses from calves vaccinated via the IN route to the calves in the other treatment groups. Calves in the IN2 and IN70 groups were always handled and vaccinated after the calves in the control, SC2, and SC70 groups; and calves in the IN2 and IN70 groups, along with their dams, were separated from the rest of the herd for 2 weeks after vaccination. Thus, it seems unlikely that BHV1, or any virus, was transmitted from the calves in the IN2 and IN70 groups to the calves in the other treatment groups. Results of other studies^{8,9,14} indicate that parenteral administration of MLV vaccines to calves does not result in transmission of vaccine strains of virus to commingled unvaccinated control calves; therefore, calves in the SC2 and SC70 groups were not separated from the calves in the control group after vaccination.

Calves in all 5 treatment groups of the present study responded to the booster vaccinations administered SC at a median age of 217 days (weaning) and 231 days, as evidenced by multiple measures of the im-

mune response, including the 4-fold increase in BVDV1 SNA titers and significant increases in the percentage of total PBMCs that expressed CD25; IFN- γ secretion by PBMCs after stimulation with BHV1; PBMC proliferation after stimulation with BHV1, BVDV1, and *P multocida*; and the percentage of PBMCs that stained positive for CD25 in addition to CD4, CD8, or WC1 (to identify $\gamma\Delta$ T cells) in blood samples obtained from calves at a median age of 262 days, compared with those obtained from calves at a median age of 217 days. Conversely, the booster vaccinations appeared to have no effect on CD25 expression by large (activated) PBMCs; the percentage of large PBMCs that expressed CD25 did not differ significantly between samples that were obtained from calves at a median age of 217 and those obtained from calves at a median age of 262 days.

In the present study, the mean BVDV1 SNA titers for calves in the SC70 group were significantly higher after administration of the priming vaccination, compared with those for calves in the control and other treatment groups. However, the mean BVDV1 SNA titer for calves in the SC70 group did not differ significantly from that for the calves in the control group in samples obtained from calves at a median age of 262 days. Because the vaccination given at a median age of 217 days represented a booster vaccine for SC70 calves and a priming vaccine for calves in the control group, it is possible that the mean BVDV1 SNA titer for calves in the SC70 group was higher than that for calves in the control group after vaccination at a median age of 217 days but before 231 days. Unfortunately, it was not possible to obtain samples from calves between the vaccinations administered at a median age of 217 and 231 days.

Occasionally, calves vaccinated against or exposed to respiratory pathogens develop a measurable CMI response but not a measurable humoral immune response^{21,22}; therefore, in the present study, multiple measures of CMI were evaluated for a subset of calves in an effort to identify any effects elicited by the various strategies for priming vaccination. Because there was no significant association between treatment group and any of the respective CMI outcomes evaluated, it would appear that route of administration of and age of calf at priming vaccination do not have an effect on CMI. These results are consistent with those of another study,³ in which vaccination of calves that had maternal antibodies with an MLV vaccine had no effect on CD25 expression by PBMCs after stimulation with BHV1 or BRSV 3 months after vaccination.

The changes detected in CMI responses after stimulation with BHV1, BVDV1, or BRSV in all groups during the observation period of the present study were apparently real because the percentage of total PBMCs that expressed CD25 in cell culture media only (background response) for samples obtained from calves at a median age of 2 days did not differ significantly, compared with the background response for samples obtained from calves at a median age of 262 days. Thus, the increase in the percentages of total and large PBMCs that expressed CD25 after stimulation with BHV1, BVDV1, and BRSV in samples obtained from calves at a median age of 262 days, compared with those in sam-

ples obtained from calves at a median age of 2 days, was apparently caused by the development of immunologic memory in response to both the priming and booster vaccinations.

Generally, after receiving the priming vaccination, the calves of the present study failed to develop a detectable response for most of the immune function variables evaluated regardless of the route of vaccine administration or the age of the calves at administration. The lack of detectable immune responses was most likely associated with the fact that the calves were only vaccinated once prior to weaning. In some studies¹²⁻¹⁴ in which results indicated a beneficial effect of vaccinating calves that have maternal antibodies, calves were vaccinated twice before weaning. In another study,²³ the mean SNA titer against *Histophilus somni* at weaning for calves that received 2 doses of a bacterin containing *H somni* and *M haemolytica* prior to weaning was significantly higher than that for unvaccinated control calves, whereas the mean SNA titer against *H somni* at weaning for calves that received only 1 dose of the bacterin prior to weaning did not differ significantly from that for unvaccinated control calves. However, results of other studies³⁻⁵ suggest that a single dose of a multivalent MLV vaccine containing BVDV2 administered to calves that have maternal antibodies can induce protective immunity for calves against challenge exposure to virulent BVDV2 several months after vaccination. The vaccine used in one of those studies⁵ was the same 7-way MLV vaccine used in the present study.

The age of calves or the concentration of maternal antibodies present at the time of priming vaccination can also impact subsequent responses, and these variables may have affected the response of calves to booster vaccination in this study. Other investigators³ reported that vaccination of calves that had maternal antibodies against BVDV slowed the decay of BVDV SNA titers and increased CMI responses against BVDV as measured via CD25 expression and production of IFN- γ and interleukin 4 by PBMCs in those calves, but the responses were dependent on the age of the calves and the concentration of maternal antibodies in the calves at the time of vaccination. For example, in that study,³ calves vaccinated at 1 to 2 weeks of age did not develop a detectable B-cell memory response, whereas calves vaccinated at 4 to 5 weeks or 7 to 8 weeks of age did develop a detectable B-cell memory response. In another study,⁶ vaccination of calves that had maternal antibodies at 3 to 8 days of age with a multivalent MLV vaccine IN did not provide protection against virulent BRSV challenge exposure 4.5 months after vaccination. Conversely, vaccination of calves that had maternal antibodies at 3 weeks of age with a modified-live BRSV vaccine IN did provide protection against virulent BRSV challenge exposure 2 months after vaccination.² Thus, the immune response of young calves with maternal antibodies following vaccination has varied among research studies.

The present study had several strengths and weaknesses. The strengths included the use of a large number of calves raised in a conventional field setting and the evaluation at multiple time points of multiple measures of both humoral immunity and CMI against several common respiratory pathogens of cattle for which

the calves had been vaccinated. The weaknesses included the use of in vitro measures of immune function to evaluate the effectiveness of vaccination rather than resistance to disease after experimental challenge or natural exposure to specific pathogens. Because the cow-calf operation on which the present study was conducted was managed with the intention of optimizing animal health, it was not feasible to induce disease in calves via challenge exposure to the various pathogens. The study herd was well managed, which benefited the present study in that it was easy to collect the required samples and administer the vaccines to the appropriate calves at the appropriate times; however, it also meant that the respiratory disease morbidity rate for calves was low historically. More significant differences may have been identified among the treatment groups of the present study had the risk of natural exposure to respiratory pathogens been greater or had the calves been experimentally challenged with a particular pathogen.

The present study was a large-scale effort to evaluate the impact of calf age at initial, or priming vaccination, and administration route of that priming vaccination on the humoral immune and CMI responses after a booster vaccination administered at weaning in beef calves raised in a conventional field setting. Results suggested that the immune response of calves following administration of a 7-way MLV booster vaccination containing BHV1, BVDV1, BVDV2, BRSV, PI3V, *M haemolytica*, and *P multocida* SC at weaning was not affected by the age at which calves received the priming vaccination or the route (IN vs SC) by which that priming vaccination was administered. However, under the conditions of the present study, SC administration of a 7-way MLV vaccine to 70-day-old calves that had maternal antibodies did induce SNA titers against BVDV1 that should have been protective against disease had those calves been subsequently exposed to BVDV1 between the time of priming vaccination and weaning.

- a. Onset 5 IN, Intervet-Schering/Plough, Summit, NJ.
- b. Once PMH SQ, Merck Animal Health, Summit, NJ.
- c. Vista Once SQ, Merck Animal Health, Summit, NJ.
- d. BD, Franklin Lakes, NJ.
- e. Identi-Plug, Jaece Industries Inc, North Tonawanda, NY.
- f. Lymphocyte separation medium, Mediatech Inc, Manassas, Va.
- g. Rabbit anti-bovine IgG (whole molecule) antibody, Sigma-Aldrich, St Louis, Mo.
- h. IgG from bovine serum, Sigma-Aldrich, St Louis, Mo.
- i. Rabbit anti-bovine IgG (whole molecule)-peroxidase antibody, Sigma-Aldrich, St Louis, Mo.
- j. Bovine IgA ELISA Quantitation Set, Bethyl Laboratories Inc, Montgomery, Tex.
- k. Provided by Merck Animal Health, Summit, NJ.
- l. Albumin from bovine serum, Sigma-Aldrich, St Louis, Mo.
- m. Sheep anti-bovine IgA, peroxidase conjugated antibody, Bethyl Laboratories Inc, Montgomery, Tex.
- n. Bovine IFN-gamma DuoSet, R&D Systems Inc, Minneapolis, Minn.
- o. Mouse anti-bovine CD25:RPE monoclonal antibody, AbD Serotec, Oxford, England.
- p. Mouse anti-bovine CD4:FITC monoclonal antibody, AbD Serotec, Oxford, England.
- q. Mouse anti-bovine CD8:FITC monoclonal antibody, AbD Serotec, Oxford, England.
- r. Mouse anti-bovine WCI:FITC monoclonal antibody, AbD Serotec, Oxford, England.

- s. Accuri C6, Accuri Cytometers Inc, Ann Arbor, Mich.
 t. Stata, version 11.0, StataCorp LP, College Station, Tex.

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