Mucosal immune response in newborn Holstein calves that had maternally derived antibodies and were vaccinated with an intranasal multivalent modified-live virus vaccine

Kevin L. Hill, DVM; Breck D. Hunsaker, DVM, PhD; Hugh G. Townsend, DVM, MSc; Sylvia van Drunen Littel-van den Hurk, PhD; Philip J. Griebel, DVM, PhD

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Design—Randomized controlled clinical trial.

Animals—23 newborn Holstein bull calves.

Procedures—Calves received colostrum and were assigned to group A (unvaccinated control calves), group B (IN vaccination on day 0), or group C (IN vaccination on days 0 and 35). Serum and nasal secretion sample (NSS) titers of antibodies specific for bovine herpesvirus 1, bovine viral diarrhea virus 1, and bovine viral diarrhea virus 2; WBC counts; and NSS interferon concentrations were determined up to day 77.

Results—Calves had high serum titers of maternally derived antibodies specific for vaccine virus antigens on day 0. High IgA and low IgG titers were detected in NSSs on day 0; NSS titers of IgA decreased by day 5. Group B and C NSS IgA titers were significantly higher than those of group A on days 10 through 35; group C IgA titers increased after the second vaccination. Serum antibody titers decreased at a similar rate among groups of calves. Interferons were not detected in NSSs, and calves did not develop leukopenia.

Conclusions and Clinical Relevance—IN vaccination of newborn calves with high concentrations of virus-neutralizing antibodies increased NSS IgA titers but did not change serum antibody titers. Revaccination of group C calves on day 35 induced IgA production. Intranasal vaccination with a modified-live virus vaccine was effective in calves that had maternally derived antibodies. (J Am Vet Med Assoc 2012;240:1231–1240)

Newborn calves are at high risk for gastrointestinal and respiratory tract infection, and inadequate transfer of maternal antibodies is an important factor that contributes to risk of disease in these calves. There is a need to enhance immune-mediated protection against infection in newborn calves, but the opinion of many clinicians and investigators is that parenteral vaccination of newborn calves is not effective. This opinion has been challenged by findings of a study indicating vaccination of 2- to 5-week-old calves that have circulating maternally derived antibodies in-Mucosal immune response in newborn Holstein calves that had maternally derived antibodies and were vaccinated with an intranasal multivalent modified-live virus vaccine

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some authors have suggested that a low number of circulating B lymphocytes may limit antibody production following vaccination of newborn calves. However, immune responses are not induced in blood, and the number of circulating blood leukocytes does not correspond with the cellular composition of lymphoid tissues. Newborn mice have low production of antibodies, which has been attributed to a low circulating concentration of C3. Similarly, newborn calves have lower circulating concentrations of C3 and other complement components than do adult cattle. The circulating concentration of C3 required for production of antibodies by B lymphocytes and the impact that C3 has on activation of B lymphocytes are not known. However, vaccination of fetal calves during the last third of gestation results in immune memory and induction of antibody production that are similar to those in adult cattle. Therefore, the immune system in fetal calves seems to be functional despite B-cell lymphopenia and low circulating concentrations of C3 in those calves.

The finding that fetal calves are immunocompetent indicates an appropriate vaccination protocol should induce protective immunity in newborn calves. However, there are contradictory reports as to whether vaccination of calves that have circulating maternally derived antibodies induces production of antibodies and protection against disease. Circulating maternally derived antibodies prevent the induction of protective immunity in 2-week-old calves after IM vaccination with a modified-live BVDV-1 vaccine. Circulating maternally derived antibodies inhibit induction of a humoral immune response after IM vaccination of calves with a live or killed BRSV vaccine. Other authors reported that circulating maternally derived antibodies prevent induction of protective immunity in newborn calves after IN vaccination with a multivalent MLV BRSV vaccine. In addition, circulating maternally derived antibodies interfere with induction of antibody production in newborn calves after IN administration of infectious BHV-1. In that study, BHV-1–specific T lymphocytes were detected in blood samples obtained from calves after IN administration of virus; however, induction of mucosal antibody production was not evaluated. Results of these studies have been interpreted by some investigators as evidence that circulating maternally derived antibodies inhibit replication of viruses or that protective immunity may not be induced in calves with immature immune systems.

Other authors reported that circulating maternally derived antibodies do not interfere with development of protective immunity if a live virus vaccine is administered to calves via the respiratory tract. Intranasal vaccination with modified-live PI-3 and BRSV vaccines induces protective immunity in 3-week-old calves that have circulating maternally derived antibodies. Intranasal vaccination of newborn calves with a multivalent MLV vaccine reduces clinical signs of disease following challenge with BHV-1, BRSV, BVDV-1, BVDV-2, and PI-3 versus clinical signs in unvaccinated control calves; however, investigators in that study used colostrum-deprived calves. Therefore, IN administration of an MLV vaccine appears to be an effective vaccination method in newborn calves, but questions remain as to whether maternally derived antibodies interfere with induction of immune responses after vaccination of calves.

The mucosal portion of the immune system provides the first defense barrier against 90% of pathogenic organisms. Commensal microflora rapidly colonize the gastrointestinal tracts of neonates after they are born. Therefore, it is not surprising that the mucosal portion of the immune system is well developed in ruminants in utero. Newborn lambs have the capacity to mount an immune response to mucosal vaccination via the small intestine and oral cavity. These findings are consistent with findings of other studies conducted to investigate the use of mucosal vaccines in newborns of a variety of domestic animal species. For example, IN vaccination of young puppies with Bordetella bronchiseptica and oral vaccination of newborn chicks with Salmonella organisms induce protective immunity against those pathogens. Therefore, we wanted to investigate whether IN vaccination with an MLV vaccine would induce mucosal production of antibodies in newborn calves that had circulating maternally derived antibodies.

Our hypothesis was that IN vaccination would induce a mucosal immune response in newborn calves that have circulating maternally derived antibodies. This hypothesis was predicated on the assumptions that maternally derived antibodies (which are primarily IgG subclass 1 in cattle) are not transported across mucosal epithelium of the upper respiratory tract, vaccine viruses establish productive infections in the upper respiratory tract, and vaccine virus antigen is amplified to a concentration that induces a mucosal immune response in calves. Concentration of dimeric IgA is <10% the concentration of IgG in colostrum of cows, and IgA may be transported across mucosal surfaces. Therefore, we measured maternally derived IgA and IgG in nasal secretions of calves before they were vaccinated and after IN administration of a commercially available multivalent MLV vaccine to determine whether IN vaccination induces endogenous production of IgA and development of immune memory in the upper respiratory tracts of calves.

Materials and Methods

Animals—Twenty-three 1-day-old Holstein bull calves were purchased over an 18-day period from a commercial dairy farm and enrolled in the study in blockwise fashion (total of 3 blocks). Dams of the calves had received an MLV vaccine 30 days before their expected day of breeding and a multivalent killed virus vaccine 60 days before their expected day of parturition; vaccines administered to the dams included BHV-1, BVDV-1, BVDV-2, PI-3, and BRSV antigens. A blood sample (10 mL) had been collected from each calf before it was fed colostrum and serum concentrations of antibodies against BVDV had been determined with a plaque inhibition assay. Colostrum had been collected from each cow, and calves received 4 L of colostrum within 6 hours after birth and an additional 4 L of colostrum 12 hours after birth. Skin biopsy samples had been obtained from an ear of each calf and submitted for immunohistochemi-
cal analysis to detect BVDV antigen. Calves that were seropositive for antibodies against BVDV (congenital infection) and calves that had positive results for BVDV in skin biopsy samples (persistent infection) were not included in the study. Calves that qualified for inclusion in the study were transported to the research facility within 24 hours after they received colostrum. All experimental procedures involving live animals were reviewed and approved by the Intervet/Schering-Plough Technical Services Committee for Respiratory Research.

Animal housing and care—Each calf was housed in a pen (1.2 X 4.9 m). Pens were separated by solid walls (height, 1.5 m). Water was provided ad libitum, and commercial milk replacer was provided twice per day to calves until they were 60 days old. A mixed-grain concentrate feed (rolled corn and barley) was offered free choice to calves after they were 2 weeks old. After they were weaned from milk replacer, calves were fed a mixed-grain concentrate feed ration and free-choice alfalfa hay.

Study design—Within 24 to 48 hours after arrival at the research facility, a blood sample (10 mL) was collected from each calf; serum was obtained, and serum samples were submitted to a diagnostic laboratory for determination via zinc turbidity assay of the total serum IgG concentration (as a measure of maternally derived antibody concentration). Twenty-three calves that had a serum IgG concentration ≥ 800 mg/dL were enrolled in the study.

Calves were randomly assigned to 1 of 3 groups; group A calves (n = 8) were not vaccinated, group B calves (8) were vaccinated once (day 0), and group C calves (7) were vaccinated twice (days 0 and 35). Day 0 was defined for all calves as the day on which group B and C calves received their first vaccination. All calves were 3 to 8 days old on day 0. A commercially available MLV vaccine that contained BHV-1, BVDV-1, BVDV-2, PI-3, and BRSV in a glicerin carrier diluent was administered IN to group B calves (on day 0) and group C calves (on days 0 and 35) in accordance with manufacturer’s instructions. Control (group A) calves were housed a sufficient distance (> 0.5 m) from vaccinated calves and were not offered free choice to calves after they were 2 weeks old. After they were weaned from milk replacer, calves were fed a mixed-grain concentrate feed ration and free-choice alfalfa hay.

A blood sample (10 mL) was collected in evacuated glass tubes that contained EDTA from each group A, B, and C calf immediately before group B and C calves were vaccinated (day 0) and on days 2, 3, 5, 7, 9, 10, 21, 35, 38, 40, 42, 45, and 56 for determination of WBC count. The WBC counts were used to detect whether IN vaccination was associated with leukopenia in calves. A blood sample (10 mL) was collected in plastic serum tubes from each group A, B, and C calf immediately before group B and C calves were vaccinated (day 0) and on days 21, 35, and 56; serum was prepared and submitted to a diagnostic laboratory for determination via plaque inhibition assay of VN antibody titers specific for BHV-1, BVDV-1, and BVDV-2.

Nasal secretion samples were obtained from each group A, B, and C calf immediately before group B and C calves were vaccinated (day 0) and on days 3, 5, 10, 21, 35, 38, 40, 42, 56, and 77. The nasal passage from which samples were collected was alternated on each sample collection day to minimize irritation of nasal mucosa. For each calf, a nostril was moistened with an aerosol spray of saline (0.9% NaCl) solution; a cotton tampon then was inserted into that nasal cavity and left in place for 20 minutes, and NSSs were collected by manual compression of tampons. Nasal secretion samples were centrifuged at 2,000 X g for 5 minutes, and the supernatant was collected. Nasal secretion samples were allocated into 500-µL aliquots, 50 µL of 10-x-protease inhibitor cocktail was added to each aliquot, and aliquots were stored at −20°C until analyzed. Enzyme-linked immunosorbent assays were performed to determine NSS titers of IgA and IgG specific for BHV-1, BVDV-1, and BVDV-2 and concentrations of IFN-α and IFN-γ. Nasal secretion samples obtained on days 0 and 56 were submitted to a diagnostic laboratory for determination of VN titers of antibodies specific for BVDV-1, BVDV-1, and BVDV-2.

Calves were monitored visually each time they were fed by an animal health technician who recorded physical condition, alertness, feed and water consumption, and presence of feces for 7 days after vaccination. A physical examination was performed on each calf by a licensed veterinarian (BDH) once per day during the study, and rectal temperature and respiration rate were recorded. Calves were treated if clinical signs of disease were detected. No adverse effects attributable to vaccination were detected in the calves. However, 1 calf from each of the treatment groups died during the study. A necropsy was performed on each calf by a veterinarian (BDH); acidosis and bloat were diagnosed in 2 calves and nephropathy and uremia attributable to urolithiasis were diagnosed in 1 calf. These calves were removed from the study and excluded from all data analyses. Therefore, for all data analyses, group A had 7 calves, group B had 7 calves, and group C had 6 calves.

IFN ELISAs—Enzyme-linked immunosorbent assays were performed as previously described to determine concentrations of IFN-α20 and IFN-γ24 in NSSs of calves. Briefly, polystyrene microwell plates were coated with monoclonal capture antibodies (mouse anti-bovine IFN-γ antibody [clone 2-2-1; 1:8,000 dilution] or mouse anti-bovine IFN-α antibodies [clones IFN-A2 and IFN-A4, 1:1 ratio; 1:1,000 dilution] in carbonate coating buffer [15mM Na2CO3, and 33mM NaHCO3; pH 9.6]) by incubation at 4°C for 16 hours. Plates were washed with TBST. Ten serial 2-fold dilutions of recombinant bovine IFN-γ and IFN-α (starting concentrations, 2,000 pg/mL; diluted in TBST containing 0.5% gelatin) were prepared for use as standards for the ELISAs. Nasal secretion samples were diluted (8 serial 2-fold dilutions in TBST containing 0.5% gelatin); 100 µL of diluted standards and NSSs were added to plate wells, and plates were incubated at 20°C for 90 minutes. Plates were washed, detection antibody (rabbit anti-bovine IFN-γ antibody [1:5,000 dilution]) or rabbit anti-bovine IFN-α antibody [1:4,000 dilution]) was added to each well, and plates were incubated at 20°C for 60 minutes. Plates were washed, secondary antibody (biotinylated goat anti-rabbit IgG); 1:10,000
IgA and IgG ELISAs—Determination of IgG and IgA titers specific for BHV-1, BVDV-1, and BVDV-2 in NSSs was performed as described previously. Briefly, 96-well polystyrene microtiter plates were incubated overnight (16 hours) at 4°C with 0.05 μg (volume, 100 μL) purified recombinant gD (BHV-1 ELISA plates), 4 ng (volume, 100 μL) recombinant E2.1 (BVDV-1 ELISA plates), or 4 ng (volume, 100 μL) recombinant E2.2 (BVDV-2 ELISA plates) per well. Plates were washed with TBST, incubated for 2 hours at room temperature (20°C) and washed. For detection of IgG specific for gD, E2.1, and E2.2, replicate plates were incubated with biotin-conjugated rabbit anti-bovine IgA (1:10,000 dilution) at 20°C for 60 minutes. Plates were washed and incubated with streptavidin-AP (1:10,000 dilution) at 20°C for 60 minutes. Plates were washed and incubated with p-nitrophenyl phosphate at 20°C for 15 minutes (plates coated with E2.1 and E2.2) or 120 minutes (plates coated with gD). Absorbance was measured at 405 nm with a reference wavelength of 490 nm. Antibody titers were expressed as the reciprocal of the highest NSS dilution that yielded an absorbance value that exceeded the mean + 2 SDs absorbance value for 3 replicates of an NSS that had negative ELISA results for gD, E2.1, and E2.2; this NSS had been collected from an unvaccinated 6-week-old Holstein calf and stored in the laboratory of one of the authors (PJG) during development of the ELISA.

Statistical analysis—Statistical analyses were performed with statistical software. To control for repeated measures, data for each calf were summed over time, and sums of the data were ranked (to account for nonnormal distribution of the summed data). Differences among data for groups of calves were determined via ANOVA on ranks. Assumptions of the ANOVA were verified by determination of equality of variances (Bartlett test), normality of residuals (Wilk-Shapiro test), and constancy of variance of residuals (residual plots) of data. Differences among means of the ranks were determined via the Tukey test for comparison of means. Spearman rank correlations were calculated to determine relationships among age of calves, serum VN titers, nasal IgA titers, and magnitude of mucosal immune response (maximum IgA ELISA titers) following vaccination. Values of P < 0.05 were considered significant.

Results

Transfer of maternally derived antibodies—Before they were fed colostrum, calves had serum VN antibody titers < 2 for BHV-1, BVDV-1, and BVDV-2. Calves had high serum VN antibody titers specific for BHV-1 (median, 128; range, 128 to 256), BVDV-1 (median, 1,024; range, 512 to 8,192), and BVDV-2 (median, 1,024; range, 256 to 4,096) on day 0 (after calves were fed colostrum but before they were vaccinated; Figure 1). These results indicated that dams had antibodies against BHV-1, BVDV-1, and BVDV-2 and that maternal antibodies against these viruses had been transferred to calves via colostrum. Serum VN antibody titers specific for BHV-1, BVDV-1, and BVDV-2 were not significantly different among groups of calves on any day of the study. A 50% decrease in serum VN titers specific for BHV-1 and BVDV-1 but not BVDV-2 was detected within 21 to 35 days after the first vaccination.

The IgG ELISA titers specific for gD, E2.1, and E2.2 were low in NSSs collected from calves on day 0 (Figure 2) and were not significantly different from IgG titers specific for these glycoproteins in NSSs collected from calves on any day after vaccination. Because NSS IgG ELISA titers remained low, titers were not determined for NSS samples collected after day 45. In contrast, IgA ELISA titers specific for gD, E2.1, and E2.2 were ≥ 10-fold higher than IgG ELISA titers specific for these glycoproteins in NSSs collected from calves before they were vaccinated on day 0 (Figure 3).

Mucosal immune response to IN vaccination—To determine whether maternally derived antibodies in NSSs obtained from calves on day 0 (before vaccination) could neutralize virus infectivity, VN assays were performed. Virus-neutralizing antibodies specific for BHV-1 were present in NSSs obtained on day 0 from calves in each group, but titers were low (Figure 4); VN titers of anti–BHV-1 antibodies were approximately 32 times as low in NSSs obtained on day 0 as they were in serum samples obtained on day 0 (Figure 1). Similarly, VN titers of antibodies specific for BVDV-1 and BVDV-2 were low in NSSs obtained from calves on day 0; VN titers specific for BVDV-1 and BVDV-2 were approximately 16 times as low in NSSs obtained on day 0 as they were in serum samples obtained on day 0. The IgA ELISA titers specific for gD were substantially lower in NSSs obtained from calves on day 5 versus titers in samples obtained on day 0 (Figure 3). Titers of IgA specific for gD were substantially higher in NSSs collected from vaccinated (group B and C) calves on day 10 than they were in samples collected from those calves on days 5, 21, or 35. Titers of IgA specific for gD were significantly (P < 0.001) higher in NSSs obtained on day 10 from vaccinated (group B and C) calves versus NSSs obtained from control (group A) calves on that day. The increase in gD-specific IgA titers in group B and C calves after the first vaccination was transient, and IgA titers specific for gD were similar in NSSs collected on days 5 and 35 from calves (ie, titers in samples from group B and C calves had returned to prevaccination titers by day 35).
The IgA ELISA titers specific for E2.1 and E2.2 were substantially lower in NSSs obtained from calves on day 5 versus titers in samples obtained on day 0 (Figure 3). The IgA ELISA titers specific for E2.1 and E2.2 were significantly (P < 0.001) higher in NSSs obtained from...
group B and C calves than they were in samples obtained from control (group A) calves on days 21 and 35; IgA ELISA titers specific for E2.2 were also significantly higher in NSSs obtained from group B and C calves on day 10 versus those obtained from group A calves on day 21.
that day. Titers of IgA specific for E2.1 and E2.2 seemed to gradually increase in group B and C calves after vaccination on day 0; IgA titers in these calves were higher on day 33 than they were on days 5 through 21.

The gD-specific IgA ELISA titers of group C calves seemed to rapidly increase after they received their second vaccination (Figure 3). The IgA ELISA titers specific for gD were significantly ($P < 0.001$) higher in NSSs obtained from group C calves versus samples obtained from group A (control) and group B calves on days 38, 45, 56, and 77. The gD–specific IgA titers of group B calves appeared to remain high after day 35, even though these titers were not significantly different from those of group A calves during that period.

The E2.1-specific IgA ELISA titers were significantly ($P < 0.001$) higher in NSSs obtained from group C calves after they received their second IN vaccination (days 45, 56, and 77) than they were in samples obtained from group A (control) calves on those days. The E2.1-specific IgA titers of group B calves appeared to remain high after day 35, even though they did not receive a second vaccination. However, E2.1-specific IgA titers for group B calves were not significantly different from titers for group A (control) calves during that period. The NSS VN antibody titers specific for BVDV-1 and BVDV-2 were not significantly different among groups of calves on day 56 (Figure 4). The gD-specific IgA titers of group B calves seemed to increase after day 35, although these titers were not significantly different from those of group A calves during that period.

An analysis was performed to determine whether age of calves or VN antibody titers on day 0 had an effect on IgA production after IN vaccination. Mean age of calves, median NSS IgA ELISA titers, and median VN antibody titers in NSSs and serum were not significantly (for all comparisons, $P \geq 0.4$) different among the groups of calves on day 0. Age of calves on day 0 was not significantly (for all comparisons, $P \geq 0.4$) related to NSS IgA ELISA titers or VN antibody titers on day 0. Age of calves on day 0 was not significantly (for all comparisons, $P \geq 0.3$) related to magnitude of mucosal immune response (ie, maximum IgA ELISA titer specific for gD, E2.1, or E2.2) after vaccination of group B and C calves. Nasal secretion sample IgA ELISA titers (specific for gD, E2.1, or E2.2) and VN antibody titers (specific for BHV-1, BVDV-1, and BVDV-2) on day 0 were not significantly (for all comparisons, $P \geq 0.2$) related to the magnitude of mucosal immune responses (ie, maximum IgA ELISA titer) specific for these antigens.

**IFN production and WBC counts**—Interferon-α and IFN-γ were not detected in NSSs of calves. The sensitivity of the ELISAs to detect IFN was 8 pg of IFN/mL (as determined by assay of recombinant bovine IFN calibration standards; data not shown). Therefore, it was determined that concentrations of IFN in NSSs were below the limits of the ELISAs to detect them.

No significant differences in WBC count were detected among groups of calves during the study (Figure 5). Similarly, when WBC counts for each calf were normalized relative to their WBC count on day 0 (to minimize the impact of variation of WBC counts on results of data analysis), there were no significant differences among groups of calves.

**Discussion**

Administration of live virus vaccines to the lower or upper respiratory tracts of newborn calves can provide protection against disease, but there is controversy as to whether circulating maternally derived antibodies interfere with induction of antibody production after vaccination. In these studies, results of which indicated maternally derived antibodies interfere with induction of immune responses, concentrations of serum antibodies were determined. However, concentrations of maternally derived antibodies or vaccine virus–specific IgA in upper or lower respiratory tract secretions of calves were not determined. Immunglobulin isotype A is selectively transported across the mucosal epithelium in cattle, and dimeric IgA, the predominant form of IgA in blood, is transported into mucosal secretions. Immunglobulin isotype G subclass 1 is the predominant immunoglobulin in colostrum of cattle, but dimeric IgA is also present in colostrum of calf at concentrations as high as 40 mg/mL. Therefore, maternally derived IgA may be present in nasal secretions of newborn calves and interfere with induction of immune responses to live virus vaccines. In the study reported here, we confirmed (by use of VN assays) that calves had circulating maternally derived antibodies specific for virus antigens to which dams had been vaccinated. In contrast, extremely low titers of maternally derived IgG specific for those vaccine virus antigens were detected (via ELISAs) in NSSs of calves. Maternally derived IgA was detected in NSSs obtained before vaccination of calves. Median VN antibody titers specific for BHV-1, BVDV-1, and BVDV-2 were approximately 16 to 32 times as low in NSSs as in serum samples obtained from calves on day 0 in the present study. This difference between nasal secretion and serum sample VN antibody titers is consistent with results of other studies indicating that < 10% of total antibody in colostrum is IgA and intact IgG is not transported.
into mucosal secretions of cattle. A lack or very low concentrations of maternally derived IgG in secretions of the upper respiratory tract may dramatically reduce interference of these antibodies with induction of an immune response after vaccination of calves with IN MLV vaccines.

The VN antibody titers specific for BHV-1, BVDV-1, and BVDV-2 were low in NSs relative to titers in serum samples in the present study. A rapid decrease in mucosal IgA specific for those viruses was detected in calves within 3 days after vaccination. This finding is consistent with the reported 2.5-day half-life of serum IgA in newborn calves and may explain contradictory results reported by authors of other studies in which IN vaccination of newborn calves was investigated. The age of a newborn calf at the time of its first vaccination may have an effect on whether there is a high enough concentration of maternally derived mucosal IgA in that calf to interfere with induction of an immune response to vaccination via a mucosal route. However, statistical analysis of data in the current study did not reveal a significant association between endogenous production of IgA in calves and age of calves or VN titers in NSs collected at the time of vaccination. The rapid decrease in titers of maternally derived IgA in the calves in the present study was followed by a substantial increase in titers of maternally derived IgA in the calves in the present study. There may be several reasons why the courses of the immune responses specific for BVDV and BHV-1 were so different in these calves. The BVDV antigens persist in antigen-presenting phagocytes in lymphoid and nonlymphoid tissues in calves. The gradual increase in BVDV-1 (E2.1)–specific IgA titers detected during 5 weeks after group B and C calves received their first vaccination and the sustained elevation of BVDV-1–specific IgA titers detected in group B calves (that received a single vaccination) for the duration of the study may have been attributable to persistence of antigen in antigen-presenting phagocytes. Similarly, BVDV-2 (E2.2)–specific IgA titers gradually increased after the first vaccination of group B and C calves, but the IgA titers for group B calves (that received a single vaccination) decreased after 35 days. To determine whether antigen persistence is a key factor in the kinetics of the mucosal immune response to vaccination against BVDV, future studies should be conducted to compare antigen persistence following vaccination against BVDV-1 and BVDV-2 in calves. Alternatively, the delay in achievement of peak IgA titers after the first vaccination against BVDV in calves in the present study may have been attributable to suppression of the immune system by vaccine viruses. Primary BVDV infections are associated with immune suppression and susceptibility to secondary infections. Results of a study in which investigators used colostrum-deprived calves indicate there is protective immunity against infection with BHV-1 and BVDV for 3 to 4 weeks following a single IN vaccination with a vaccine containing modified-live BHV-1, BVDV-1, and BVDV-2.

Production of IgA increased in group C calves following their first IN vaccination, and there was a further increase in the magnitude and duration of IgA production after their second IN vaccination, on day 35. This anamnestic response indicated that immune memory was induced in these calves and that the induction of endogenous IgA production after the first vaccination did not interfere with induction of a secondary mucosal immune response. It is important to mention that IN vaccination and induction of a local mucosal IgA response did not prevent a decrease in titers of serum VN antibodies in these calves. This finding is consistent with results of other studies in which IN vaccination of newborn calves does not substantially delay a decrease in titers of maternally derived antibodies in calves. The failure of IN vaccination to prevent a decrease in serum VN antibody titers in calves in the other studies was interpreted as evidence that mucosal vaccination was not effective. However, results of the present study suggested that a mucosal immune response can develop after IN vaccination without substantial change in serum antibody titers. Therefore, monitoring of local mucosal IgA production rather than serum antibody titers may be a more effective way to assess the magnitude of a mucosal immune response following IN vaccination. Furthermore, determination of the duration of a mucosal immune response after vaccination may be the best way to determine the optimal vaccination schedule for achieving immune protection following use of an IN vaccine.

Avirulent MLV vaccines, such as BHV-1 and PI-3, are known to activate innate immune responses such as production of IFN; these innate immune responses provide protection against respiratory infections by other viruses. Therefore, the absence of detectable concentrations of IFN in NSs after IN vaccination of calves in the present study was surprising. This may have been attributable to a low rate of replication of viruses or an immature developmental state of the innate immune system in these newborn calves. The absence of significant changes in WBC counts in calves in the present study is consistent with the absence of a detectable IFN response because leukopenia has been detected following administration of recombinant bovine IFN-α to calves in another study. Interferon-γ plays a key role in enhancing presentation of antigens and has been shown to increase IgA production in cattle. Therefore, the absence of detectable concentrations of IFN at mucosal surfaces in newborn calves may limit the magnitude of immune responses at those locations. Future investigations may be warranted to determine whether recombinant IFN-γ, which is a potent adjuvant in cattle, could enhance mucosal immune responses in newborn calves.

Results of the present study indicated that maternally derived IgA but not IgG was transported across the mucosal epithelium of the upper respiratory tract and that titers of maternally derived IgA decreased rap-
idly in nasal secretions of calves. Detection of IgA in NSs of calves indicated that IN delivery of a multiva- lent MLV vaccine was an effective strategy to induce primary and secondary mucosal immune responses in calves that had high concentrations of maternally de- rived antibodies. Mucosal IgA production was induced without significant changes in serum antibody titers in these calves. This finding indicates the importance of monitoring mucosal immune responses when evaluating the immunogenicity of an IN vaccine.

References

31. STATISTIX, version 7.0, Analytical Software, Tallahassee, Fla.