Effect of passive immunity on the development of a protective immune response against bovine viral diarrhea virus in calves

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Objective—To determine whether passively acquired antibodies prevent development of a protective immune response to live virus in calves.

Animals—18 calves.

Procedures—Calves were caught immediately after birth and tested free of bovine viral diarrhea virus (BVDV) and serum antibodies against BVDV. Within 48 hours, 12 calves were fed colostrum that contained antibodies against BVDV and 6 calves received BVDV antibody free milk replacer. Three milk replacer fed and 6 colostrum fed calves were exposed to virulent BVDV2-1373 at 2 to 5 weeks of life when passively acquired serum antibody titers were high. After serum antibody titers against BVDV had decayed to undetectable concentrations (at 7 to 9 months of age), the 3 remaining milk replacer fed calves, 6 colostrum fed calves previously exposed to BVDV2-1373, and 6 colostrum fed calves that had not been exposed to the virus were inoculated with BVDV2-1373.

Results—Passively acquired antibodies prevented clinical disease in inoculated colostrum fed calves at 2 to 5 weeks of life. Serum antibody titers did not increase in these calves following virus inoculation, and serum antibody titers decayed at the same rate as in noninoculated colostrum fed calves. Inoculated colostrum fed calves were still protected from clinical disease after serum antibody titers had decayed to nondetectable concentrations. Same age colostrum fed calves that had not been previously exposed to the virus were not protected.

Conclusions and Clinical Relevance—A protective immune response was mounted in calves with passive immunity, but was not reflected by serum antibodies titers. This finding has implications for evaluating vaccine efficacy and immune status. (*Am J Vet Res* 2003;64:65–69)

Bovine viral diarrhea virus (BVDV), a member of the pestivirus genus within the family Flaviviridae,[1] is an economically important pathogen of cattle found worldwide. Both modified live and killed BVDV vaccines are available. Mechanisms by which these vaccines induce a protective response are as yet undefined. Neutralizing antibody responses are measurable in cattle after inoculation with either modified live or killed vaccines.[2-6] Whether these vaccines also induce cell-mediated immunity is largely unknown. Bovine viral diarrhea virus vaccination programs have 2 goals; 1 is the prevention of in utero infections (fetal infections), and the other is the prevention of clinical disease in calves and adult cattle (postnatal infections). Until recently, most acute postnatal BVDV infections were considered to be clinically mild, and emphasis has been placed on preventing fetal infection. However, the recognition of severe acute forms of bovine viral diarrhea[7-10] has resulted in renewed interest in the prevention of postnatal infection. Vaccination of suckling calves against BVDV is desirable from management and disease control standpoints. There is a question, however, as to whether vaccination is negated by the presence of maternal antibodies. For this reason, it is generally recommended that vaccination be performed after the disappearance of maternal antibodies (ie, at 6 to 8 months of age).[11] Unfortunately, this leaves a window of opportunity for acute infections as maternal antibodies decay. The purpose of the study presented here was to determine whether active protective immunity would develop in calves with passive immunity.

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Materials and Methods

Viruses—Virulent BVDV2-1373 was used in virus inoculations. To determine viral neutralizing antibody titers in colostrum and sera, BVDV2-1373 and BVDV1-NY-1 were used. Viral stocks were propagated as described previously.[12] Methods used to determine viral titers were also described in an earlier publication.[3] The BVDV-NY-1 is a standard laboratory strain from the BVDV1 genotype. The BVDV2-1373[a] belongs to the BVDV2 genotype. The BVDV1 and BVDV2 genotypes have been declared distinct species within the pestivirus genus.[1] The BVDV2-1373 was isolated from an outbreak of severe acute BVDV in Ontario, Canada.[7] Both viruses were noncytopathic as determined by activity in cell culture[13] and the production of the NS3 polypeptide.[14]

Colostrum—Nine pregnant cows were vaccinated twice, with a killed BVDV1 and BVDV2 vaccine,[b] at 8 and 4 weeks prior to their calving dates. Milk from the first (colostrum) and second (first milk) milkings after calving were collected and immediately frozen at –20°C. The colostrum and first milk samples were later pooled, divided into 2,800-mL aliquots, and frozen at –20°C until used. Neutralizing antibody titers, in the colostrum pool and the first milk pool, against type 1 (BVDV1-NY-1) and type 2 (BVDV2-1373) BVDV were determined as described previously.[3] Calves born to these cows were not used in our study.

Calves—Calves of both sexes and various breeds were caught immediately after birth. Calves were determined to be free of BVDV and antibodies against BVDV. Blood samples and skin specimens were obtained within 2 hours of birth. Serum samples were tested for antibodies against type 1 and type 2 BVDV. Freeze-thaw lysates of buffy coat samples were passed twice on bovine turbinate cells. Cells were then stained with a polyclonal serum prepared by hyperimmunizing a steer with BVDV1, BVDV2, and border disease virus (antigenically related virus from the pestivirus genus). Skin specimens (ear notches) were snap frozen in isopentane and immunohistochemistry performed by use of monoclonal antibodies reactive against the E2 protein.[15] Calves were fed and observed for clinical signs of bovine viral diarrhea twice per 24-hour period. For the first 6 weeks of life and for 3-week intervals following viral exposure, calves were housed in individual pens in a confinement facility. When not housed in individual pens, calves were housed in pens with other calves from the same experimental group.

Experimental design—Calves (n=18) were placed into 4 experimental groups (**Appendix**). Group-A (n = 6) and -B (6) calves were fed pooled colostrum for 3 feedings and pooled first milk for the fourth feeding during the first 48 hours after birth. Feedings were 2,800 mL in volume and administered via a stomach tube. Group-C (n = 3) and -D (3) calves served as controls to assess viral virulence. Group-C and -D calves were fed four 2,800-mL feedings of milk replacer,[c] which had been tested free of antibodies against BVDV, in the 48 hours following birth. Milk replacer feedings were also delivered via a stomach tube in 2,800-mL volumes. Group-A and -C calves were exposed to BVDV2-1373 at 2 to 5 weeks of age. Group-A calves were challenged with a second exposure of BVDV2-1373 at 7 to 9 months of age when serum neutralizing antibody titers had declined to < 1:8. Group-B calves were only exposed once to the virus. This was at 7 to 9 months of age when serum neutralizing antibody titers had declined to < 1:8. Group-D calves were exposed to the virus at 7 to 9 months. Blood samples were collected at least monthly from all calves to determine serum viral neutralizing antibody titers.

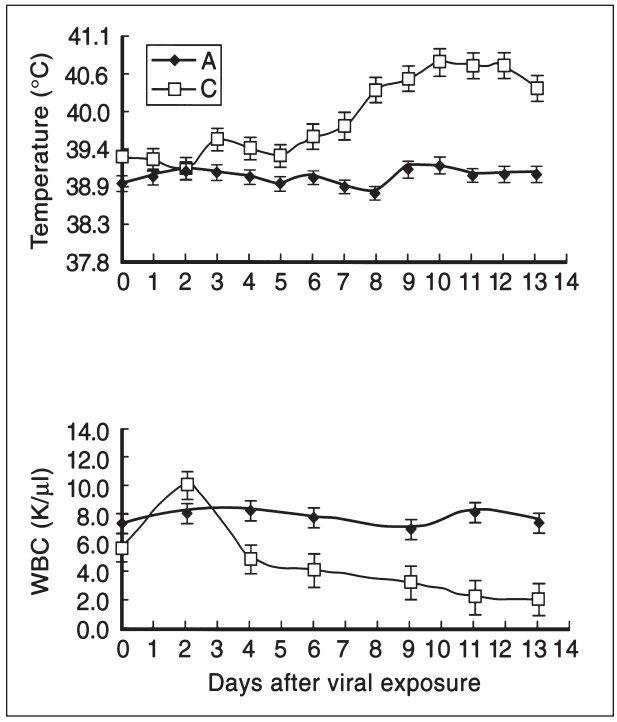

Figure 1—Mean (± SEM) basal rectal body temperatures (top panel) and WBC counts (bottom panel) in calves versus days after exposure to virulent bovine viral diarrhea virus (BVDV2-1373). Group-A calves were fed colostrum that contained antibodies against BVDV in the first 48 hours following birth. Group-C calves were fed milk replacer that did not contain antibodies against BVDV.

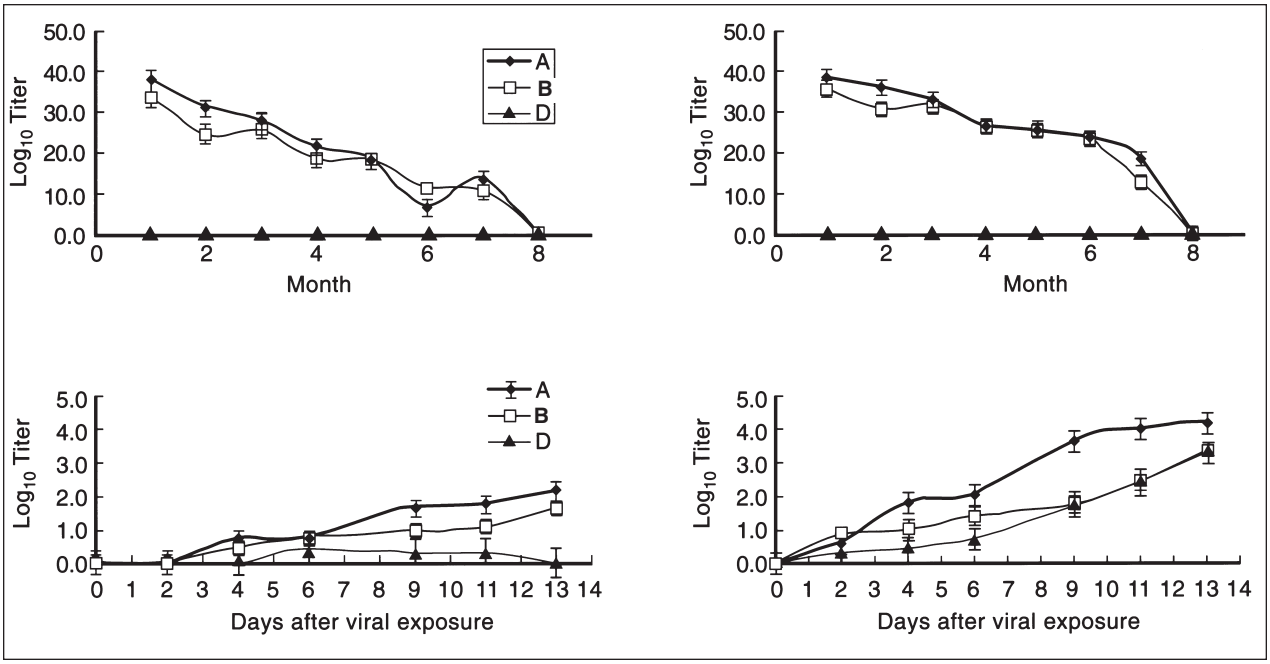

Figure 2—Changes in mean (± SEM) serum neutralizing antibody titers against BVDV1-NY-1 (left panels) and BVDV2-1373 (right panels) in calves from 1 month until before exposure to virus at 7 to 9 months of age (top panels) following exposure to virus at 7 to 9 months of age (bottom panels). Group-A and -B calves were fed colostrum. Group-D calves were fed milk replacer. Group-A calves were exposed to BVDV-1373 at 2 to 5 weeks of age and again at 7 to 9 months of age. Group-B and -D calves were only exposed at 7 to 9 months of age.

Calves were exposed to virus by placing 2.5 mL of freeze-thaw lysate, prepared from cultured bovine cells infected with BVDV2-1373, in each nostril (5 mL total). All lysates used as inoculum had a titer of 10^6 tissue culture infectious $dose_{50}$/mL. After exposure to virus, basal rectal body temperature was determined daily (days 0 through 16 after exposure). Serum[d] and buffy coat[e] samples were obtained from blood samples collected on days 0, 2, 4, 6, 9, 11, and 13 after exposure for determination of neutralizing antibody titers and virus isolation, respectively. In addition, blood samples were collected in buffered citrate[f] (0.129M, 3.8% solution) on days 2, 4, 6, 9, 11, and 13 after exposure to determine WBC and platelet counts.

Serum viral neutralizing antibody titers were determined as described previously.[3] Virus isolation from buffy coat samples was performed. The buffy coat layer was separated by centrifugation[12] and put through 1 freeze-thaw cycle (–20 to 25°C). A 0.5 mL aliquot of buffy coat freeze-thaw lysate was mixed with 0.5 mL of media (minimum essential medium[g] plus 10% vol/vol fetal calf serum). The resulting mixture was used to inoculate a 25 cm^3, 60 to 70% confluent flask of Madin Darby bovine kidney cells. After rocking at 37°C for 1 hour, the inoculum was removed from the cells and replaced with 5 mL of cell culture media (Eagle medium supplemented with 10% fetal calf serum that had been tested free of BVDV and antibodies against BVDV). After 5 days the cell culture (including media) was frozen at –80°C. After thawing, 1 mL of the resulting lysate was added to a fresh 25 cm^3 flask of Madin Darby bovine kidney cells. After rocking for 1 hour at 37°C, 4 mL of cell culture media was added. After incubating for 5 days, total RNA was prepared from the culture and tested for BVDV as described.[12] Virus isolation was not attempted from nasal swab specimens because we could not differentiate between inoculum and replicated virus in these samples.

White blood cell and platelet counts were determined by use of a cytometer[h] per the manufacturer's directions. Platelet counts were only determined for the 7- to 9-month-old calves.

Statistical analysis—The mixed models analysis of variance procedure from a statistical software program[i] was used to compare group means. Differences were considered significant for P values <0.05. Mean (± SEM) group measurements of basal rectal temperature, WBC count, serum neutralizing antibody titers, and platelet counts were graphed for comparison.

Results

Antibody titers against type 1 and type 2 BVDV in the pooled colostrum were 128,000 and 4,096,000, respectively. Antibody titers in the first milk against type 1 and type 2 BVDV were 8,192 and 64,000, respectively. One week after birth, Group-A and -B calves had mean serum antibody titers of 2,211 against type 1 BVDV and 11,456 against type 2 BVDV. Group-C and -D calves did not have measurable serum antibodies against type 1 or type 2 BVDV.

Group-A calves were protected from acute disease when exposed to virulent type 2 BVDV in the first 3 to 5 weeks of life. Group-A calves did not have an increase in basal rectal temperature or a decrease in WBC counts (**Fig 1**). Serum titers of neutralizing antibodies in group-A calves did not increase after exposure to virus. Group-C calves exposed to BVDV2-1373 had a mean rectal temperature that was > 40.6°C on days 10 through 12. In addition, group-C calves had a mean decrease in WBC counts of > 60% by day 11. Group-C calves had diarrhea with anorexia and were unsteady when trying to walk or stand by day 12. By day 13 group-C calves became moribund and were euthanatized. The euthanasia protocol was as follows: calves were first tranquilized with xylazine hydrochloride (0.1 mg/kg) and then euthanatized with 26% solution of sodium pentobarbital (2 mL for the first 4.5 kg plus 1 mL for each additional 4.5 kg).

The log difference in serum neutralizing titer against BVDV1 versus BVDV2 observed at 1 week was not seen at 1 month. Serum titers of neutralizing antibodies in group-A and -B calves declined at similar rates (**Fig 2**). When serum antibody titers were < 1:8, group-A and -B calves were exposed to BVDV2-1373. In the period between the first and second exposure to virus, a group-A calf died of causes unrelated to BVDV infection. In addition, age-matched, colostrum-

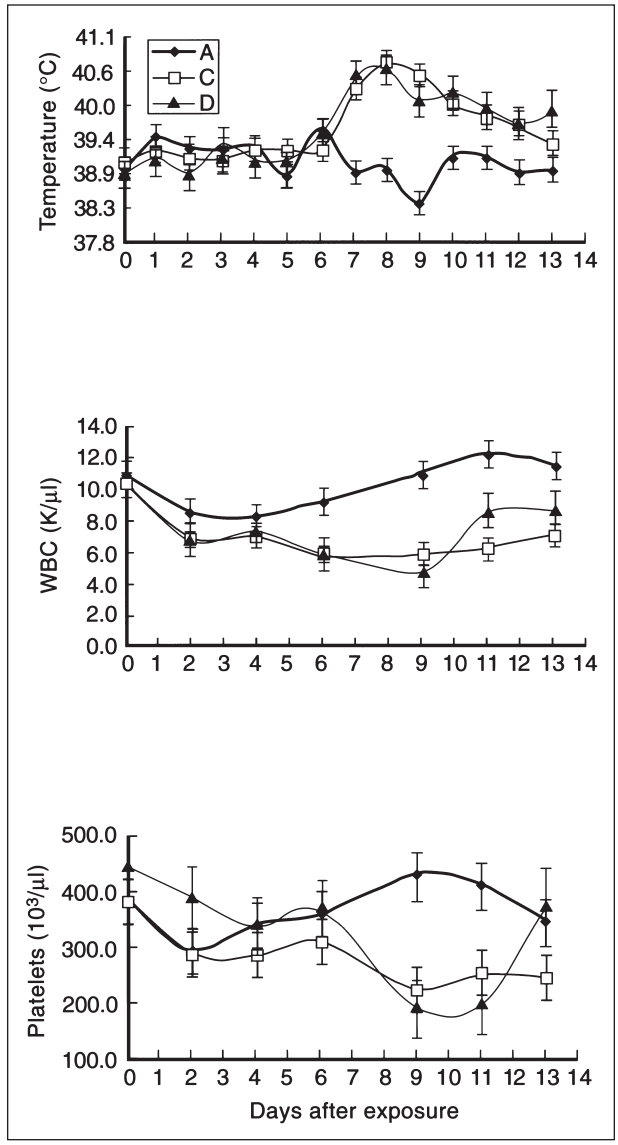

Figure 3—Mean (± SEM) basal rectal body temperatures (top panel), WBC counts (middle panel), and platelet counts (bottom panel) in calves versus days after exposure to BVDV2-1373 at 7 to 9 months of age. Group-A and -B calves were fed colostrum. Group-D calves were fed milk replacer. Group-A calves were exposed to BVDV-1373 at 2 to 5 weeks of age and again at 7 to 9 months of age. Group-B and -D calves were only exposed at 7 to 9 months of age.

deprived group-D calves were also exposed to the virus. When exposed to the virus at 7 to 9 months, group-A calves did not have an increase in rectal temperature or a decrease in WBCs or platelet counts (**Fig 3**). In contrast, group-B and -D calves had a mean 0.6°C increase in rectal temperature. The decrease in WBCs and platelet counts in group-B and -D calves ranged from 40 to > 60%. During the 2-week period following viral exposure, group-A calves had a mean body weight gain of 7.7 kg. In contrast, group-B calves had a mean body weight loss of 18.1 kg, and group-D calves had a mean body weight loss of 53.3 kg. Serum antibody titers after viral exposure at 7 to 9 months increased to higher concentrations in group-A calves, compared with group-B and -D calves (mean 33,843 vs 5,504). Virus was not isolated from the buffy coat samples of group-A calves after virus exposure. Between days 2 and 13 after virus challenge, virus was isolated from the buffy coat samples from all group-B and -D calves.

Discussion

In our study, calves provided with passively acquired antibody protection did not become clinically ill when exposed to a highly virulent BVDV at 2 to 5 weeks of life. These calves remained clinically normal when challenged with the same virulent virus even after passively acquired serum antibody titers had decayed to undetectable concentrations. The protection afforded by colostrum, with a high neutralizing antibody titer against BVDV, fed in the first 48 hours of life was not unexpected. Previous research has shown that calves protected by low to intermediate serum titers of passively acquired antibodies do not develop clinical signs of disease when exposed to a virulent BVDV.[16,17] More surprising was protection from clinical disease observed in the calves of our study during the second challenge after serum antibody titers had waned. The lack of clinical disease is evidence of an active protective response that was mounted in calves with passive immunity. This response was not reflected by detectable serum antibody titers. However, there did appear to be an anamnestic response in these calves, because serum antibody titers increased to higher concentrations than in control calves. An explanation for these observations is that the presence of passively acquired serum antibodies reduced but did not eliminate viral replication after the first challenge. The limited viral replication, which was not sufficient to cause clinical disease, led to the development of a weak B lymphocyte response and a protective T lymphocyte response.

The mechanisms by which vaccination reduces clinical disease associated with BVDV infection is poorly understood. Previously, it has been shown that systemic virus neutralizing antibodies are involved in the prevention of postnatal infections.[16] Our results suggest that cell-mediated immunity may also play a major role in preventing postnatal disease.

Protection in the absence of detectable serum antibody titers has implications for evaluating the immune status of animals and for measuring vaccine efficacy. At present, a criterion for licensing BVDV vaccines is the development of serum antibody titers. Our research suggests that protection may exist in the absence of detectable serum antibodies. Serum antibody titers may be an inadequate measure of vaccine protection. Further, it is standard practice for researchers to screen cattle to be used in BVDV studies for previous exposure to pestiviruses on the basis of the presence of serum antibodies against BVDV. Results of our study indicate that screening on the basis of serum antibodies may not accurately reflect the immune status of cattle. This may explain discrepancies in viral virulence sometimes observed between animals that confound researchers.

[a]Provided by Dr. Susy Carman, Animal Health Laboratory, University of Guelph, Ontario, Canada.
[b]Provided by Fort Dodge Animal Health, Fort Dodge, Iowa.
[c]Arbie Feeds, Marshalltown, Iowa.
[d]Vacutainer SST gel and clot activator tubes, Becton Dickinson, Franklin Lakes, NJ.
[e]Vacutainer sodium heparin tubes, Becton Dickinson, Franklin Lakes, NJ.
[f]Vacutainer buffered citrate Na (9:10), Becton Dickinson, Franklin Lakes, NJ.
[g]Life Technologies Inc, Rockville, Md.
[h]HV 1500, CDC Technologies Inc, Oxford, Conn.
[i]SAS Proprietary Software Release 8.2, SAS Institute Inc, Cary, NC.

Appendix

Experimental design

Calf groups	Received colostrum	Virus exposure and age: BVDV2-1373 (2–5 weeks old)	Virus exposure and age: BVDV2-1373 (7–9 months old)
A (n =6)	Yes	Yes	Yes
B (6)	Yes	No	Yes
C (3)*	No	Yes	No
D (3)*	No	No	Yes

*Control groups.
BVDV = Bovine viral diarrhea virus.

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