Onset of Protection from Experimental Infection with Type 2 Bovine Viral Diarrhea Virus Following Vaccination with a Modified-Live Vaccine*

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INTRODUCTION

Humoral and cell-mediated immune responses are known to be important in the protection against viral infections. In addition, although pathogen- and antigen-specific humoral and cell-mediated responses occur, the general innate immune response is also very important, especially during the acute or initial phase of viral replication and pathogenesis. The innate immune response and the development of a specific immune response following vaccination can be measured using several methods. Humoral response to vaccination against viruses is most commonly measured through the determination of serum virus neutralization antibody titers after vaccination. The development of antibody titers can generally be detected as early as 7 to 10 days after vaccination. Cell-mediated responses are more difficult to measure. Methods such as lymphocyte proliferation assays, T cell cytotoxicity assays, and cytokine assays can provide measurements of the

CLINICAL RELEVANCE

The onset of protection after the administration of a modified-live bovine viral diarrhea virus (BVDV) vaccine was determined. Protection was determined following experimental infection with a virulent type 2 BVDV (strain 1373) in cattle vaccinated 3, 5, or 7 days before BVDV infection. Protection, as measured by reduced virus shedding, lack of leukopenia, reduction in viremia, and reduced mortality, was present as early as 3 days after vaccination with a single dose of modified-live BVDV vaccine. Complete protection was obtained in cattle vaccinated 5 or 7 days before BVDV experimental infection.
BVDV-associated disease primarily occurs when weaned calves are marketed and commingled as stocker cattle and into the feedyards. In these environments, the ability to provide rapid, protective immune responses is essential. Modified-live vaccines generally provide faster immune responses than killed virus vaccines through virus replication that stimulates the innate immune response.

The objective of this experimental study was to characterize the response to modified-live BVDV vaccine immediately after administration. The purpose of the study was to determine whether a modified-live BVDV vaccine can elicit a protective response to BVDV infection within 72 hours after vaccination. This was accomplished by measuring specific responses to vaccination and by using an experimental BVDV challenge to determine the ability of vaccination to protect against BVDV infection at various postvaccination intervals.

A significant level of protection was obtained in animals that were vaccinated 5 or 7 days before experimental challenge.

MATERIALS AND METHODS

Animals

Forty bulls (8 to 9 months of age) were used in the study. All animals were determined not to be persistently infected with BVDV by immunohistochemistry of ear notch biopsies. In addition, all animals were seronegative against BVDV (<1:5) as determined by serum neutralization assay to both type 1 and type 2 BVDV reference viruses. All animals were clinically normal at the onset of the study. Animals were randomly assigned to four treatment groups of 10 animals each using a random number generator (Research Randomizer, www.randomizer.org).
Study Design
A group of 10 animals consisted of a treatment group. Vaccinations (Vx) were administered as follows:

- **Day –3 Vx Group:** Vaccinated 3 days before BVDV experimental challenge
- **Day –5 Vx Group:** Vaccinated 5 days before BVDV experimental challenge
- **Day –7 Vx Group:** Vaccinated 7 days before BVDV experimental challenge
- **Nontreated Control Group:** Animals were not vaccinated

**Vaccination**
The vaccine administered was a USDA-licensed stock material released for sale (Express 5, Boehringer Ingelheim). The Express 5 vaccine is a modified-live combination viral vaccine containing BVD type 1a (Singer strain) and type 2a (296 strain), bovine rhinotracheitis (IBR), parainfluenza3 (PI3), and bovine respiratory syncytial virus (BRSV). A single 2-ml dose was administered subcutaneously using a sterile 18-guage × 1-inch needle for each animal according to manufacturers’ recommendations. Only one dose of vaccine was given at the designated time according to treatment group before BVDV experimental challenge.

**Virus Challenge**
All animals were experimentally infected with a noncytopathic type 2 BVDV isolate 1373 obtained from J. Ridpath, National Animal Disease Center (Ames, IA). Challenge inoculum consisted of an infected cell culture supernatant at 5 × 10⁵ cell culture 50% infective doses (CCID₅₀)/ml of virus. The experimental intranasal viral infection was done by aerosolization of 5 ml of inoculum using a DeVilbiss aerosolizer (DeVilbiss, Somerset, PA) and a vacuum pump.

**Sampling**
Samples collected on postchallenge days 0, 4, 6, 7, 8, 10, 12, and 14 included whole blood, nasal swabs, and clotted blood for serum. Body temperatures were also recorded on postchallenge days 0, 4, 6, 7, 8, 10, 12, and 14. Individual body weights were obtained for all calves on postchallenge days 0, 6, 7, and 14 using an electronic scale.

**Observation**
Animals were observed daily; clinical scores and rectal temperatures were recorded for each calf on days 0 and 4 through 14 (no data was collected on days 1 to 3). Clinical signs—depression, dyspnea, nasal discharge, ocular discharge, appetite, coughing, and diarrhea—were assigned a numeric value based on severity and were recorded on a clinical assessment form. Individuals observing the daily clinical signs after challenge were blinded to the group assignments of individual animals.

**Laboratory Analysis**
Total leukocyte counts and platelet counts were conducted using an automatic Coulter counter at the Auburn University College of Veterinary Medicine clinical pathology service laboratory. Leukocyte and platelet counts were recorded as numbers/cc. γ-Interferon levels were determined by ELISA on serum collected on postchallenge days 0, 4, 7, and 12 (IFN-Gamma ELISA, BioSource [Invitrogen], Carlsbad, CA).

**Virus Isolation**
Virus isolation was done on nasal swabs, serum, and buffy coat cell preparations. Virus isolation was conducted using Marden–Darby bovine kidney cells and 25 cm² cell culture flasks or 96-well tissue culture plates supplemented with 10% horse serum. Virus isolation was determined by detection of viral antigen
by immunoperoxidase staining using a BVDV-specific monoclonal antibody.

**Necropsy**

Complete necropsies, including histopathologic examination of H&E fixed tissues, were performed on all animals that died or were euthanized during the course of the study. Gross examinations were performed within 12 hours of death.

**Statistical Analysis**

Statistical analysis was conducted using JMP 5.1 Statistical Discovery Software, (SAS Institute, Cary, NC). Analysis of variance and mean comparisons for all pairs was accomplished using Tukey–Kramer honestly significant difference (HSD). Statistical significance was determined at $P < .05$.

**RESULTS**

Postchallenge determination of virus titers in the challenge inoculum indicated that the virus titer of the inoculum was $4.0 \times 10^3$ CCID$_{50}$/ml. During challenge, 5.0 ml of BVDV strain 1373 was inoculated into each animal on day 0. There were no observed postvaccination complications or evidence of clinical signs in any of the 30 vaccinated and 10 nonvaccinated animals.

Body temperature varied among treatment groups, but the difference was not measurable. Clinical scores were higher in the nonvaccinated control animals but were not significantly increased ($P > .05$).

Day 0 and postchallenge day 14 body weights were compared between treatment groups (Figure 1). The mean differences in weights of the nonvaccinated, day –3 Vx, day –5 Vx, and day –7 Vx groups from day 0 to day 14 were –34, 1, 25, and 20 lb, respectively. There was a significant difference in mean weights between the nonvaccinated control group and the –5 Vx and –7 Vx groups ($P < .05$). There was no significant difference between the nonvaccinated control group and the day –3 Vx group ($P > .05$).

BVDV was isolated from serum, nasal swabs, and leukocytes after experimental infection. Virus was isolated from at least one sample type (serum, leukocytes, or nasal swab) from all animals in the nonvaccinated control group on at least one day during the 14 days after challenge (Figures 2 and 3). The ability to isolate virus from the animals vaccinated 3, 5, or 7 days before experimental infection was significantly reduced compared with the nonvaccinated controls for days 6, 7, and 8 ($P < .05$; Figure 3). BVDV was isolated from nasal

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![Figure 1. Body weights of animals in the various treatment groups determined on day 0 and postchallenge day 14. Asterisks indicate treatment groups that were significantly different from the nonvaccinated control group ($P < .05$).](image-url)
swabs collected from all nonvaccinated control animals (Figure 2). Animals in the –3Vx group had limited virus isolation positive samples during the sampling period: Only four of 10 animals had a positive nasal swab during the sampling period.

Mean total leukocyte counts were compared between treatment groups during the postchallenge sampling period (Figure 4). On postchallenge day 6, a transient leukopenia, which is characteristic for acute BVDV infection, was observed in the nonvaccinated control group. The characteristic leukopenia was not observed in any of the vaccinated groups, with the exception of two animals vaccinated on day –3 (these animals died); in addition, virus was isolated from nasal swabs and leukocytes from these same two animals. On postchallenge day 6, there was a significant difference in the mean leukocyte counts between the nonvaccinated animals and all the vaccinated treatment groups (Figure 4). On postchallenge days 7 and 8, there was a significant difference in mean leukocyte counts between the nonvaccinated treatment group and –5 Vx and –7 Vx groups. A significant difference in mean leukocyte count between the nonvaccinated treatment group and the –3 Vx group occurred only on postchallenge day 6.

Platelet counts were compared between treatment groups during the postchallenge sampling period (Figure 5). There were no significant differences between the mean platelet counts among the different treatment groups.

γ-Interferon levels in serum samples were determined by ELISA and recorded as optical
density values using a 450 nm filter (OD$_{450}$). Duplicate values were determined for each sample, and an average value for each sample was obtained for postchallenge sample collection days 0, 4, 7, and 12. Mean OD$_{450}$ values were compared between the four different treatment groups (Figure 6); no trends or significant changes in values between treatment groups were detected.

By postchallenge day 14, six of 10 nonvaccinated control animals died (n = 5) or required euthanasia (n = 1). By postchallenge day 14, two of 10 animals in the –3 Vx group died (n = 1) or required euthanasia (n = 1). None of the animals in the –5 Vx and –7 Vx groups died or were euthanized. Necropsies were performed on all animals that died or were euthanized. Gross lesions included oral mucosal ulcerations and mild ulcerations of the mucosa of the gastrointestinal tract, including the esophagus. Lungs were normal to mildly congested in appearance, with no evidence of bact-

Figure 4. Comparison of mean leukocyte counts for the different treatment groups during the postchallenge sampling period. Note leukopenia occurring at day 6 in the nonvaccinated control group. Asterisk indicates significant difference between mean counts at day 6 (P < .05).
In the present study, intranasal aerosolization of the 1373 isolate induced acute disease and a resulting systemic BVDV infection. Gross and histopathologic examination did not reveal secondary pneumonia. Death losses were primarily due to BVDV systemic infection.

Evaluation of clinical pathology data indicated that a transient leukopenia typical of acute BVDV infection occurred. However, in the present study, the type 2 isolate did not induce thrombocytopenia as has been reported previously, generally in younger animals. Experimental reproduction of thrombocytopenia has been achieved in colostrum-deprived calves. It is interesting to note that the severe clinical disease and mortality that occurred following the experimental infection did not begin until approximately 12 days after infection. Laboratory data, such as viremia (detected in leukocytes, nasal swabs, and serum) and leukopenia (as determined by leukocyte counts), suggest that the disease peaked approximately 6 to 8 days after experimental infection. This type of pattern is typical of a bi-phasic febrile response of viral diseases. This delayed effect has not been the typical pattern of BVDV-induced diseases previously reported. It may be reasonable to speculate that the initial leukopenia was related to the primary phase of BVDV replication and that the subsequent demand created the severe lymphoid depletion seen histologically in most lymphoid tissues. Lymphoid depletion has been reported to occur in severe acute BVDV infections and has also been reported in “classical” mucosal disease. It is likely that severe lymphoid depletion is associated with acute death. Ridpath and associates have reported that the lymphocytopenia of BVDV isolates in vitro correlates with virulence. Further characterization of the pathogenesis of the lymphoid depletion following BVDV infection is needed.

To determine the effect of modified-live vaccination, various types of data were compared, including virus isolation results, clinical assessment, total leukocyte counts, and mortality. It is clear that vaccination had a positive effect on protecting animals from disease following experimental infection with the type 2 BVDV isolate 1373. From statistical evaluation of data between treatment groups, a significant level of protection was obtained in animals that were vaccinated 5 or 7 days before experimental challenge. Although there was some protection in animals vaccinated 3 days before the challenge, the difference cannot be considered significant based on mortality.

From evaluation of virus isolation from nasal swabs, vaccination completely reduced nasal
shedding of BVDV following experimental infection when animals were vaccinated 5 or 7 days before infection (Figure 3). Nasal shedding occurred in animals vaccinated 3 days before infection but was reduced compared with the nonvaccinated controls (four of 10 versus 10 of 10, respectively). The ability of vaccination to reduce nasal shedding in infected animals within days following vaccination can provide a benefit in reducing the spread of BVDV in commingled cattle. Therefore, modified-live vaccination of high-risk cattle may immediately reduce the potential for BVDV transmission within groups of cattle at risk of developing acute BVDV infections because of commingling or potential exposure to animals persistently infected with BVDV.

Current requirements, as determined by Title 9 of the Code of Federal Regulations (9 CFR), for BVDV protection are based on preventing leukopenia and the ability to isolate BVDV from blood after challenge. These requirements are based on the fact that severe clinical disease does not typically occur after experimental BVDV challenge with most isolates. The subclinical nature of BVDV infections has made it difficult to observe and quantitate the level of protection afforded by various methods of vaccination and types of vaccines. Using a challenge strain such as the type 2 1373 isolate may provide an improved method of virus challenge to demonstrate protection as a result of an increased ability to measure differences in treatment groups following experimental challenge. It is likely that the protection observed in the –5 Vx and –7 Vx groups would not have been noted if a less-virulent BVDV challenge strain had been used.

In addition, it is noteworthy that the animals that died in the –3 Vx group developed severe leukopenia that did not start to rebound until approximately 10 to 11 days after infection. These two animals were the only animals in the –3 Vx group in which BVDV was isolated from leukocytes and nasal swabs. Viremia was detected only once in two other animals in the –3 Vx group, and that was in the serum on day 6. Therefore, prevention of viremia and leukopenia may be important factors associated with the ability of vaccination to provide protection from BVDV infection.

**CONCLUSION**

Based on the results of the present study, it can be concluded that vaccination with modified-live virus vaccine immediately before virus exposure can provide benefits. Vaccination of animals with the modified-live BVDV vaccine (Express 5) 5 or 7 days before experimental infection induced rapid protection against the...
type 2 BVDV strain that resulted in severe mortality in unvaccinated animals. Vaccination 3 days before challenge provided measurable protection by preventing BVDV replication through the reduction of the level of viremia and the development of leukopenia after experimental infection. In addition, vaccination reduced the level of nasal shedding of virus following experimental inoculation.

REFERENCES