PROTECTION AGAINST BOVINE VIRAL DIARRHEA VIRUS (BVDV) OF CALVES VACCINATED WITH A BOVINE HERPESVIRUS-1 (BHV-1)-BVDV RECOMBINANT

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ABSTRACT

A recently developed recombinant bovine herpesvirus 1 (BHV-1) virus containing the envelope protein gp53 of bovine viral diarrhea virus (BVDV) type 1 was assessed for its ability to protect against BVDV in calves. Four calves were vaccinated intranasally with the recombinant BHV-1-BVDV vaccine and did not exhibit any clinical signs following vaccination. The vaccine virus was recovered from all vaccinated calves on days 8 through 10. Twenty-eight days after vaccination, the four vaccinated and four control calves were challenged with the type 1 BVDV, NY-1. All calves had slight temperature elevations but the clinical signs were more severe in the control calves. The platelet counts were depressed in the control calves. Prior to challenge, neither group had BVDV serum neutralizing antibody. Following challenge, the vaccinated calves developed higher serum antibody levels indicating a secondary immune response. Calves were euthanized and tissues were taken weeks following infection. No latent BHV-1 virus was detected from the trigeminal ganglion of any of the vaccinated calves. The recombinant BHV-1 virus vaccine containing a single BVDV protein provided partial protection against BVDV infection. This recombinant virus replication appeared to be restricted to nasal passages.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV-1) infections are a major source of respiratory and reproductive problems in cattle (Fields et al., 1996; Ludwig, 1983). Live attenuated vaccine strains of BHV-1 are effective and have been used safely in cattle for several years (Bello et al., 1992). BVDV vaccines that control persistent infections, which occurs in animals that are infected in utero and results in lifelong viral shedding, are nonexistent. Modified-live BVDV vaccines are available, but they are not safe to use in pregnant animals. Inactivated BVDV vaccines can be used in a preg-
nant animal, but they are inconvenient and ineffective at controlling persistent infections (Baker, 1995).

Bello et al. (1992) determined BHV-1 is useful as an expression and vaccine vector. Whetstone et al. (1992) determined the BHV-1 thymidine kinase (TK) gene was non-essential for BHV-1 reactivation in cattle. This led to the development of the BHV-1-BVDV recombinant virus vaccine. The glycoprotein 53 (gp53) of BVDV is the major target for neutralizing antibody against BVD virus. The gp53 from BVDV was inserted into the TK locus of a TK--BHV-1 genome. This work was done by Dr. L.J. Bello and Dr. W.C. Lawrence of the University of Pennsylvania (personal communication).

The use of the BHV-1-BVDV recombinant virus vaccine in pregnant cattle to control persistent infections of the fetuses is an important and promising endeavor. This preliminary vaccine trial using calves is a model for future trials to protect pregnant cows against BVDV infection.

MATERIALS

The vaccine virus used was the recombinant BHV-1 (v1V31) containing gp53 of the NADL strain of BVDV type 1 from the University of Pennsylvania in Philadelphia, PA. The cells used for all assays were bovine turbinate (Bt) cells. The challenge virus used was the BVDV NY-1 from the National Veterinary Services Laboratory in Ames, IA. BVD Singer type 1 and BVD A125 type 2 strains of virus were used for the serum neutralization assays which were performed by the Serology section of the Animal Disease Research and Diagnostic Laboratory (ADRDL) at South Dakota State University (SDSU).

Eight male, BHV-1 and BVDV negative, Holstein calves were used in the study. The calves were four to five months old and ranged in weight from 200 to 300 pounds. Two groups of calves were used, a vaccinated group and a control group, with each group consisting of four animals. The calves were initially housed first in an open enclosure with calf hutches and later moved to an open-front barn in a small pasture enclosure.

METHODS

The vaccinated calves were intranasally inoculated with 10⁷ TCID₅₀/2ml of recombinant BHV-1-BVDV vaccine on day 0. Nasal samples were taken day 0 to 14 to monitor for recombinant BHV-1-BVDV excretion. Clinical signs were taken every day. The clinical signs included respiration, attitude, nasal discharge, temperature, and diarrhea. The clinical signs were rated on a 0 to 4 point scale, (i.e. normal nasal discharge=0, whereas thick or crusted nasal discharge=4). Serum samples were taken every seven days starting at day 0 to monitor for seroconversion to recombinant BHV-1-BVDV.

The vaccinated and control group calves were intranasally inoculated with 10⁷ TCID₅₀/2ml of BVDV on day 28 of the study. Nasal samples were taken days 28 through 35 to monitor for BVDV excretion. Clinical signs were taken every day with the same categories and ratings as the pre-challenge period. Serum samples were taken every seven days to monitor for seroconversion to
BVDV. Whole blood was taken days 28 through 38 to count the number of whole blood cells and platelets.

Calves were vaccinated and/or challenged intranasally. The calves were secured in a headgate and halter. A small plastic biosafety bag was placed over their muzzles to induce hypoxia, and increase respiration rate and inspiration volume. The bag was removed and the vaccine/challenge was administered with a Chromist TLC aerosol unit (Gelman Sciences) for one minute to each nostril. The bag was again placed over the calves' muzzles and hypoxia was induced. The halter was removed and the calves were released.

The calves were euthanized at the end of the study. The tissues harvested were: tonsil, spleen, thymus, testes, Peyer's patches, trigeminal ganglion, and mediastinal, mesenteric, retropharyngeal, and subiliac lymph nodes.

The calves antibody levels for BHV-1 and BVDV were determined by a virus neutralization assay (Manual of Standards for Diagnostic Tests and Vaccines, 1992). The titers of the vaccine and virus were determined using the Kärber method TCID₅₀ assay (Kärber, 1931).

The nasal samples taken after vaccination and challenge were collected and virus isolations were performed. The secretions were collected by aspiration using a vacuum pump. 0.5 ml of nasal secretions were added to 4.5 ml of 1% FBS media. This mixture was vortexed, centrifuged and filter sterilized in 0.45 mm filters. Duplicates were plated at 1:5 serial dilutions on Bt cells. The BHV-1 isolation plates after vaccination were read four days after inoculation and the cytopathic effect (CPE) was recorded. Seven days after the inoculation, the cells on the BVDV isolation plates were fixed with 20% acetone, 80% PBS and 0.02% BSA. The plates were dried overnight and frozen at -70°C. Later a BVDV immunoperoxidase test was performed on the cells to detect BVDV in the samples (Saliki, 1997).

White blood cell and platelet counts were performed by the Clinical Pathology section at the ADRDL of SDSU from the whole blood.

The calves were all euthanized by electrocution and tissue samples were taken and were frozen in -80°C. Virus isolations were performed on these samples.

RESULTS

All of the calves had sero-converted to BVDV type 1 by day 56 of the study or 28 days after the challenge. Although all of the calves sero-converted, the response peak of the serological titers of the vaccinated calves was faster and more pronounced than the control calves response (Fig. 1). Calf #4 values were excluded from the data because of a high and outlying BVDV type 2 titer on a single day.

BHV-1 was recovered 8 and 10 days post-vaccination (Fig. 2). BVDV was not detected from any nasal samples after challenge (data not shown).

The post-vaccination temperatures were normal and clinical scores for post-vaccination were negligible (Fig. 3 & 4). The post-challenge temperatures of the vaccinated and control calf groups were similar (Fig. 5). The post-chal-
Figure 1. Singer “BVDV-1” and A125 “VBDV-2” average neutralizing antibody titer in sera of vaccinated and control calves.

Figure 2. Recombinant BHV-1 shedding in nasal secretions after intra-nasal inoculation.
Figure 3. Post-vaccination average calf rectal-temperature values.

Figure 4. Average post-vaccination calf clinical scores.
Figure 5. Average calf rectal temperature values 1 day pre-challenge and 10 days post-challenge with NY-1 BVDV.

Figure 6. Average calf clinical scores following challenge with NY-1 BVDV.
Figure 7. Average calf-white blood cells counts following challenge with NY-1 BVDV.

Figure 8. Average calf-blood platelets counts following challenge with NY-1 BVDV.
Challenge clinical scores showed an increase in signs during day 5 to 6 and day 8 to 9 (Fig. 6).

The white blood cell count of the vaccinated calf group remained normal throughout the trial and the control calf group's count was slightly higher and increased on day 35 (Fig. 7). The platelet count of the vaccinated calf group remained normal after challenge, while the control calf group's count was depressed (Fig. 8).

No latent BHV-1 was detected from the trigeminal ganglion of any of the vaccinated calves.

DISCUSSION

The recombinant BHV-1-BVDV was safe to use in calves. The calves showed minimal reactions to the vaccine when it was administered to them as measured by body temperatures and clinical scores. These minimal symptoms coincided with the detection of the recombinant BHV-1-BVDV shedding in the nasal secretions of the vaccinated calves (Fig. 2). This indicated that local replication occurred. Interestingly, no apparent systemic replication occurred as no latency could be detected in the trigeminal ganglia during the necropsy.

After challenge the vaccinated animals exhibited less clinical disease than the controls. The clinical signs were less severe in the vaccinated animals and the clinical pathology results indicated there was no effect on the vaccinated animals.

Serum neutralizations indicated the development of neutralizing antibody titers to BVDV type 1, but not to the BVDV type 2 (Fig. 1). This shows the specificity of type 1 gp53 for type 1 and not for BVDV type 2. After the challenge there was a significant increase in the vaccinated BVDV type 1 titers compared to the control BVDV type 1 titers. This represents a secondary immune response in the vaccinated animals, showing the vaccine had induced memory cell development to BVDV. Work continues to evaluate the nasal secretions antibody levels.

In conclusion, the calves vaccinated with recombinant BHV-1-BVDV virus vaccine showed no adverse reactions to the vaccine. The vaccine did replicate locally in the respiratory tract and there were noticeable differences in the post-challenge clinical scores, temperatures, and WBC and platelet counts of the vaccinated and control calves. The recombinant BHV-1-BVDV vaccine did provide partial protection against a BVDV challenge. This study suggests a trial with pregnant cows would provide significant information towards creating a successful vaccine against BVDV persistent infections in cattle.

ACKNOWLEDGMENTS

Special thanks to Alicia Sundet, Theodore Stier, Ausama Yousif, the Serology and Clinical Pathology sections of the ADRDL for their help. Also thank you to the Joseph F. Nelson Undergraduate Research Mentorship and the ADRDL for financial support.
REFERENCES


