

# Protection Against a Bovine Viral Diarrhea Virus (BVDV) Type 1 Challenge in 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, BHV-1 (v1V31), 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 and the replication appeared to be restricted to nasal passages. Twenty-eight days after vaccination, the four vaccinated and four control calves were challenged with the type 1 BVDV, strain NY-1. All calves had slight temperature elevations but the clinical signs were more severe in the control calves. The platelet counts were significantly depressed in the control calves. Prior to challenge, neither group had BVDV serum neutralizing antibody. The vaccinated calves developed higher serum antibody levels 2 months following challenge, indicating a secondary immune response. Necropsy was performed six 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.

## 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 BHV-1 vaccine strains are effective and have been used safely in cattle for several

years (Bello et al., 1992). BVDV vaccines fail to control persistent infections, which occur in animals that are infected in utero at 40-120 days of gestation, and result in lifelong viral shedding. Modified-live BVDV vaccines are available, but they are not safe to use in pregnant animals. Inactivated BVDV vaccines can be used in a pregnant animal, but they are inconvenient and ineffective at controlling persistent infections (Baker, 1995).

BHV-1 is useful as an expression and vaccine vector (Bello et al. 1992). Genes from other viruses can be inserted into the BHV-1 thymidine kinase (TK) gene because the TK gene is non-essential for BHV-1 replication in cattle and TK-BHV-1 viruses protect pregnant cows against abortion (Whetstone et al. 1992). This led to the development of the BHV-1-BVDV recombinant virus, BHV-1 (v1V31). 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<sup>-</sup> BHV-1 genome. This work was done by Dr. L.J. Bello and Dr. W.C. Lawrence, University of Pennsylvania (data not published).

The use of a BHV-1-BVDV recombinant virus vaccine in pregnant cattle to control both BHV-1 and BVDV abortions and BVDV persistent infections of the fetuses is an important and promising approach. This preliminary vaccine trial using calves is a model for future trials to protect pregnant cows against BHV-1 and BVDV infection. The objective of this study was to determine if the BHV-1 recombinant virus vaccine was safe and efficacious.

## Materials and Methods

*Viruses and Cells.* The vaccine virus used was the recombinant BHV-1 (v1V31) containing gp53 of the NADL strain of BVDV type 1 was

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prepared at the University of Pennsylvania in Philadelphia, PA. The cells used for all assays were bovine turbinate (Bt) cells. The cells were grown in minimum essential media (MEM) containing 5% fetal bovine serum (FBS). The challenge virus used was type 1 BVDV NY-1 from the National Veterinary Services Laboratory in Ames, IA. BVDV Singer type 1 and BVDV A125 type 2 strains were used for the BVDV type 1 and type 2 serum neutralization assays respectively.

*Animals.* 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. The study was reviewed and approved by the SDSU Institutional Animal Care and Use Committee (IACUC).

*Vaccination and Challenge Studies.* Calves were vaccinated and/or challenged intranasally. The calves were secured in a headgate and restrained using a rope halter. A small plastic biosafety bag was placed over their muzzles to induce hypoxia for 1-2 minutes to 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 vaccinated calves were intranasally inoculated with  $10^7$ TCID<sub>50</sub>/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  $10^{7.9}$ TCID<sub>50</sub>/2ml of NY-1 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 7-14 days from 0- 90 days to monitor for BHV-1 or BVDV seroconversion. Whole blood was taken days 28 through 38 to count the number of whole blood cells and platelets.

*Virus Isolation and Serology.* 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. One-half of a milliliter (0.5 ml) of nasal secretions were added to 4.5 ml of MEM containing 1% FBS. 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). The viral titers were determined using a TCID<sub>50</sub> assay (Karber, 1931).

Antibody levels for BHV-1 and BVDV were determined by a virus neutralization assay using two fold dilutions (Manual of Standards for Diagnostic Tests and Vaccines, 1992) by the Serology Section of the Animal Disease Research and Diagnostic Laboratory (ADRDL) at SDSU.

*Clinical Pathology.* White blood cell and platelet counts were performed by the Clinical Pathology section at the ADRDL of SDSU from the whole blood on days 27-38 (Days -1 to 10 post challenge).

*Necropsy.* The calves were all euthanized by electrocution at the end of the study on Day 90. The tissues harvested were: tonsil, spleen, thymus, testes, Peyer's patches, trigeminal ganglion, and mediastinal, mesenteric, retropharyngeal, and subiliac lymph nodes.

Paired tissue samples were taken, one set was fresh and one set was preserved in formalin. The fresh tissues were frozen in  $-80^{\circ}\text{C}$ . Virus isolations were performed on these samples.

*Statistical Analysis.* The results were analyzed using a student's T-test for statistical significance.

## Results

*Growth of Recombinant Virus and Clinical Signs following Vaccination.* BHV-1 was recovered from all animals from days 3-10 days post-vaccination (Figure 1). The most consistent days were days 8-10. Titers of the recombinant recovered from the nasal secretions were as high as  $10^6$  TCID<sub>50</sub>/ml.

The post-vaccination temperatures were normal (Figure 2) and clinical scores for post-vaccination were negligible (Figure 3) with a very slight increase seen from days 5-8.

*Clinical Signs and Virus Isolation Following Challenge.* BVDV was not detected from any nasal samples after challenge (data not shown). No latent BHV-1 was detected from the trigeminal ganglion of any of the vaccinated calves (data not shown)

The post-challenge temperatures of the vaccinated and control calf groups were similar (Figure 4). The post-challenge clinical scores showed an increase in signs during day 5 to 6 and day 8 to 9 with the control group scores always higher (Figure 5). However none of the differences were significant.

*Clinical Pathology.* The white blood cell (WBC) count of both the vaccinated and control calf group declined on days 1-2 post challenge (Days 29 and 30) (Figure 6). The vaccinated calves remained slightly lower than normal on days 4-6 post challenge and returned to normal on day 7. The control calves WBC count was returned to normal on day 5 post-challenge and increased significantly on day 7 ( $p < 0.10$ ) (Figure 6). The platelet count of the vaccinated calf group remained normal after challenge, while the control calf group's count was depressed significantly on days 34 ( $p < 0.1$ ), 36 ( $p < 0.001$ ) and 37 ( $p < 0.05$ ) (Figure 7).

*Serology.* The initial development and kinetics of BVDV type 1 serum neutralization response was similar between the vaccinates and control animals with the exception of the BVDV type 1 titers of the vaccinated group which were higher at day 90 (Figure 8). All of the calves seroconverted to BVDV type 1 by day 56 of the study (28 days post-challenge) (Figure 8). Interestingly none of the vaccinated calves seroconverted to BHV-1 (data not shown). The response to serotype 2 was low indicating low cross reactivity between BVDV type 1 NY-1 strain and BVDV type 2 A125 (Figure 8). The values for Calf #4 were excluded from the data because of a high outlying BVDV type 2 titer on a single day.

## Discussion

This study established that a recombinant BHV-1-BVDV was safe to use in calves. The calves showed minimal reactions to the vaccine 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 (Figure 1). 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 although none of the differences were significant ( $p > 0.10$ ). The clinical pathology results indicated a significant increase in white blood cells in the control animals on day 35 while there was no effect on the vaccinated animals. Transient leukopenia normally associated with BVDV infection did not occur in this study. There was a significant thrombocytopenia present in the control animals on days 34, 36 and 37.

Serum neutralizations indicated the development of neutralizing antibody titers to BVDV type 1, but not to the BVDV type 2 (Figure 8). This shows the specificity of type 1 gp53 for type 1 and not for BVDV type 2. After the challenge there was an increase in the vaccinated BVDV type 1 titer at 90 days post vaccination 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.

In conclusion, this small pilot study suggests a role for a recombinant BVDV-BHV-1 vaccine in the control of respiratory and reproductive disease caused by these two viruses. Calves vaccinated with recombinant BHV-1-BVDV virus vaccine showed no adverse reactions to the vaccine and the vaccine replicated locally in the respiratory tract. There were differences in the post-challenge clinical scores and type 1 BVDV serum neutralization titers and significant differences in the WBC and platelet counts between the vaccinated and control calves. The recombinant BHV-1-BVDV vaccine provided partial protection against a BVDV challenge. This study suggests a trial with pregnant cows

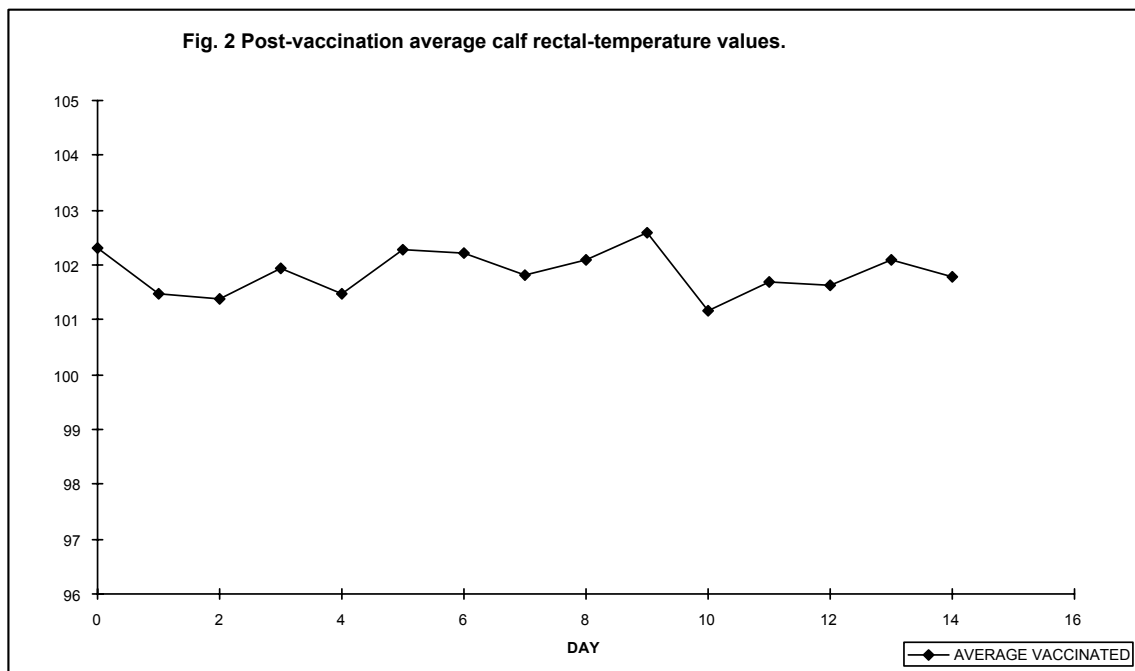
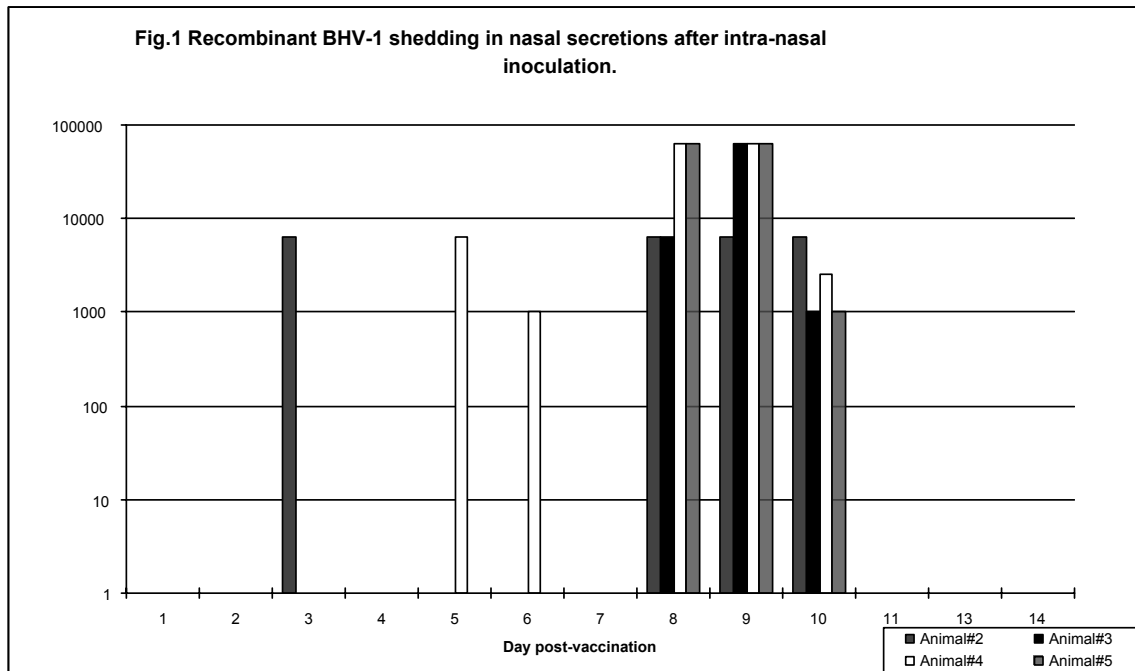
would provide significant information towards creating a successful vaccine to control both BHV-1 and BVDV abortions and BVDV persistent infections of the fetus.

### **Acknowledgements**

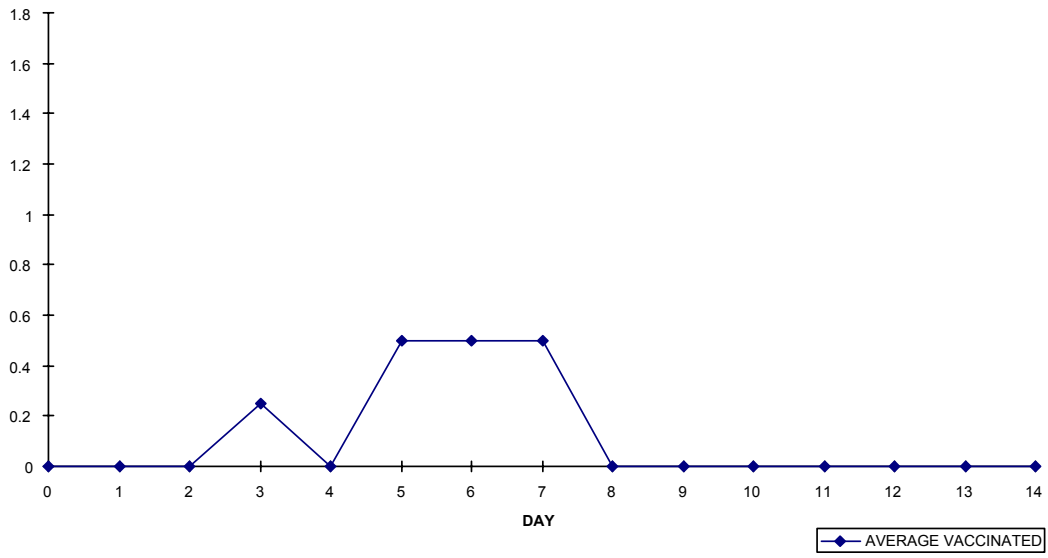
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**Fig. 3 Average post-vaccination calf clinical scores.**



**Fig. 4 Body Temperatures following BVDV Challenge**

