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Modified-Live Bovine Viral Diarrhea Virus (BVDV) Type 1a Vaccine Provides Protection Against Fetal Infection after Challenge with either Type 1b or Type 2 BVDV

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Abstract

The objective of two bovine viral diarrhea virus (BVDV) fetal protection studies was to determine if vaccination with modified-live virus (MLV) BVDV type 1a (BVDV1a) vaccine would protect fetuses from infection with BVDV type 2 (BVDV2) or type 1b (BVDV1b) virus. The experimental vaccine administered to the cows and heifers had the minimum antigen load dose of MLV BVDV1a and the full (commonly marketed) antigen dose of infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3), bovine respiratory syncytial virus (BRSV), and Leptospira Canicola-Grippotyphosa-Hardjo-Ictero-haemorrhagiae-Pomona (Lepto-CGHIP) bacterin. In Trial A, 25 pregnant vaccinated cows and heifers and 10 pregnant unvaccinated controls were challenged with BVDV2. In Trial B, 25 pregnant vaccinated cows and heifers and eight pregnant unvaccinated controls were challenged with BVDV1b. In both trials, fetuses were obtained by Cesarean section, which was performed after approximately 150 days of gestation (range 148-174 days), and presence or absence of fetal BVDV infection was determined. All control fetuses were infected with BVDV. In Trial A, all fetuses (N = 25) of vaccinated dams were free of BVDV infection. In Trial B, one cow did not have a fetus at 150 days of gestation; of 24 fetuses, 23 were negative for BVDV and one fetus was positive for BVDV. In contrast to previous studies, these results suggest that vaccination with MLV BVDV1a vaccine will significantly reduce fetal infection following challenge with BVDV2.

Keywords: bovine, BVDV, vaccine, fetus, fetal infection

Résumé

L'objectif de deux études sur la protection des fœtus contre le virus de la diarrhée virale bovine (BVDV) était de déterminer si la vaccination avec un vaccin à virus vivants modifiés (MLV) contenant le virus BVDV type 1a (BVDV1a) protégerait les fœtus contre l'infection avec le virus BVDV type 2 (BVDV2) ou le virus BVDV type 1b (BVDV1b). Le vaccin expérimental administré aux vaches et aux taures comportait la dose minimum d'antigène du BVDV1a et la dose complète d'antigène (communément mise en marché) de la rhinotrachéite bovine infectieuse (IBR), du parainfluenza-3 (PI3), du virus respiratoire syncytial bovin (BRSV) et de bactérine de *Leptospira* Canicola-Grippotyphosa-Hardjo-Ictero-haemorrhagiae-Pomona (Lepto-CGHIP). Dans l'essai A, 25 vaches et taures gestantes vaccinées et 10 vaches gestantes non-vaccinées témoins ont été provoquées avec le BVDV2. Dans l'essai B, 25 vaches et taures gestantes vaccinées et huit vaches gestantes non-vaccinées témoins ont été provoquées avec le BVDV1b. Dans les deux essais, les fœtus ont été obtenus par césarienne après approximativement 150 jours en gestation (entre 148 et 174 jours). La présence ou l'absence d'infection fœtale avec le BVDV a été déterminée. Tous les fœtus du groupe témoin étaient infectés avec le BVDV. Dans l'essai A, aucun des fœtus (N = 25) des mères vaccinées n'était infecté par le BVDV. Dans l'essai B, l'une des vaches n'avait pas de fœtus après 150 jours. Parmi les 24 fœtus restants, 23 fœtus n'étaient pas infectés avec le BVDV alors qu'un fœtus était positif. À l'opposé d'études précédentes, ces résultats suggèrent que la vaccination avec des virus vivants modifiés comportant

le virus BVDV1a réduit significativement l'infection fœtale après provocation avec le BVDV2.

Introduction

Distributed worldwide, bovine viral diarrhea virus (BVDV) has been economically detrimental since first described in the 1940s.[1,9] Bovine viral diarrhea viruses are enveloped, single-stranded RNA Pestiviruses in the Flaviviridae family that are classified as cytopathic (CP) or noncytopathic (NCP) based on biotype.[1] Investigations associated with the advent of peracute BVDV infections in Ontario, Canada in 1993-1994[13] resulted in BVDV classification as types 1a, 1b, or 2 based on genotypic variations in the 5' untranslated region,[14,16] and BVDV2 was often associated with peracute infections. These outbreaks did not correlate well with the previous concept that the more severe form of disease caused by BVDV, mucosal disease (MD), could be readily differentiated from the milder form, bovine viral diarrhea (BVD), simply by clinical outcome and lesions.[9] There was simply more variation in virulence, especially in the NCP BVDV, than had previously been recognized. Antigenic variation affected viral virulence and infectivity. One could partially blame the outbreaks on poor vaccination practices since, in the later part of 1994, the majority of Saskatchewan dairy herds were not adequately vaccinated against BVDV.[4] But in 1996, BVDV2 was isolated in 28 cow-calf herds with unusually high infertility rates, abortions and stillbirths. Nine of those herds had been properly inoculated with BVDV1a vaccine, suggesting that maternal immunization with BVDV1a had not protected fetuses from antigenically distinct genotypic strains of BVDV.[18] Cortese *et al*[5] found that MLV BVDV1 vaccination was associated with 88% fetal protection when pregnant cows were challenged with BVDV1, but Brock *et al*[2] reported only 58% fetal protection when pregnant cows previously vaccinated with BVDV1 were challenged with BVDV2.

BVD infections result in infertility, abortions, stillbirths, non-viable calves and congenital defects.[1] The continuance of BVDV in cattle is dependent on vertical transmission of the virus from dam to fetus prior to development of specific immunity. The result of fetal BVDV infection early in gestation is fetal resorption or abortion. Infecting a pregnant dam with NCP BVDV at 42 to 125 days of gestation results in the birth of persistently infected (PI) calves.[12] Calves PI with BVDV will shed the virus for life.[1] They spread virus by horizontal transmission to other susceptible animals, and later when they mature into PI dams they infect their fetus by vertical transmission. Providing fetal protection and preventing BVDV spread through PI calves is an extremely important characteristic of BVDV vaccines.

The studies described herein were used to support label claims that the vaccine[1] would aid in prevention of persistent BVDV1 and BVDV2 infection of the fetus when used as directed in the cow or heifer 30 to 60 days before breeding.

Materials and Methods

Animals

Trial A – BVDV2 challenge

We obtained a group of 36 yearling heifers from a single ranch and sent serum samples from these heifers to Colorado State University Veterinary Diagnostic Laboratory (CSU-VDL) for BVDV2 antibody serum neutralization (SN) testing, which was negative (SN titer <1:8). In addition, we utilized 14 mature cows from the University of Nebraska's BVD-free herd and sent their serum samples to the Veterinary Diagnostic Center at the University of Nebraska-Lincoln; all BVDV1 and BVDV2 antibody SN testing was negative (SN titer <1:2). Thus, at the onset of Trial A, BVDV2 antibody SN titers were negative for all 50 females, and none of the cattle were pregnant or had a history of BVDV infection or vaccination.

Trial B – BVDV1b challenge

We obtained a group of 50 adult cows from a single herd, but one was excluded on Day 0 post-vaccination (PV) because she was pregnant. For Trial B, BVDV1 antibody SN titers were negative (SN titer <1:2) for all 49 adult females, and they had no history of BVDV infection or vaccination.

Animal Housing and Care

Cattle for both trials were kept at the University of Wyoming Animal Science Research Farm, Laramie, Wyoming, in controlled outdoor facilities of sufficient size that the space per animal complied with applicable animal welfare standards. There was no direct contact with other cattle and the control group was kept separate from the vaccinate group for more than 14 days postvaccination (PV) to prevent possible control animal seroconversion due to vaccine virus exposure. Only authorized personnel worked with the animals, supplying water *ad libitum* and feeding a complete ration once daily. Appropriately trained personnel observed all study animals daily, both pre- and post-challenge, and considered all cattle acceptably healthy for virus challenge.

Test Vaccine

The test vaccine for both trials was a MLV BVDV1a Singer strain minimum antigen load with the full (commonly marketed) antigen dose of Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona (Lepto-CGHIP) bacterin, infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3), and bovine respiratory

syncytial virus (BRSV). A minimum antigen load of BVDV was utilized to comply with Center for Veterinary Biologics requirements (Title 9 of the Code of Federal Regulation 113.311 (8)(d)(3). This vaccine was formulated at Fort Dodge Animal Health Research and Development facility as a 2 mL dose.

Challenge Viruses

The NCP BVDV2 challenge virus used in Trial A, Wyoming State Veterinary Laboratory (WSVL) isolate 96B2222, was isolated from a weak, two-day-old calf from a herd in which the cows were vaccinated with a BVDV1a modified-live virus vaccine prior to breeding.[18] The NCP BVDV1b challenge virus used in Trial B, WSVL isolate 97B1415, was isolated from the tissues of a 14-day-old PI calf. Drs. Julia Ridpath and Steve Bolin of the National Animal Disease Center determined the genotype of both challenge viruses.

The viruses used for challenge were grown in bovine turbinate (BT) cells maintained in minimal essential media (MEM) + 10% horse serum (HS) + 4 mM L-glutamine[2] and determined to be free of contaminating BVDV. Flasks of BT cells were inoculated with the second passage of virus in OptiMEM[3] without serum, and incubated for 48 hours at 98.6°F (37°C). The flasks were frozen at –94°F (–70°C), thawed and the culture media pooled. Cell debris was pelleted by centrifugation and 1 mL aliquots of clarified media were stored at –94°F (–70°C). The inoculum was prepared in OptiMEM and frozen at –94°F (–70°C), and an aliquot was titrated to determine the tissue culture infectious dose 50% ($TCID_{50}$). Aliquots of the frozen inoculum were thawed just prior to inoculation of each group of animals.

The amount of virus in the virus stocks and inoculum was quantified by serial 10-fold dilutions in media. One hundred µL of each dilution was placed into each of eight wells in a 96-well plate. One hundred µL of a suspension of BT cells (1 x 10^5 cells/mL) was added to each well and the plate incubated for four days. The cells were fixed for 10 minutes in 20% acetone:80% phosphate-buffered saline (PBS) and allowed to air-dry overnight. Noncytopathic BVDV was detected using Mab 20.10.6[4] diluted 1:1600 in PBS, 0.05% Tween 20, 2.95% NaCl, biotinylated rabbit anti-mouse IgG[5] (1:500 in 60% chicken serum[6]: 40% PBS), strepavidin-horseradish peroxidase[7] (1:1000 in wash buffer), and 5 mg/mL 3-amino-9-ethylcarbazole in 0.05 M sodium acetate buffer, pH 5.0[8] + 2 L of 3% H_2O_2.[17] The wells were examined by light microscopy for cytoplasmic staining. The amount of virus was calculated using the method of Reed and Muench[15] and reported as $TCID_{50}$.

Experimental Design Protocol

The desired number of animals to be challenged in each trial was 35 total, split into two groups with 25 vaccinates and 10 controls. The number of animals initially enrolled was higher to account for less than 100% conception. There were 50 females utilized in each trial. A random number was assigned to each animal using a Microsoft Excel random number generator. Animals were ranked in ascending order and divided into vaccination and control groups. In Trial B one of the animals assigned to the control group was found to be pregnant and was not enrolled. All enrolled animals tested negative (SN <1:2) for the challenge virus, BVDV2 or BVDV1a for Trial A and B, respectively, and were subsequently injected with 2 mL of vaccine or PBS as indicated by their assignment in either the vaccinate or the control group. After injection, vaccinate and control groups were kept separate for more than 14 days to prevent any possibility of control animal seroconversion due to vaccine virus exposure.

Estrus was synchronized by initially using melengestrol acetate[9] (Trial A) or vaginal implant[10] (Trial B) and later, prostaglandin F2α[11] injection. The day of the prostaglandin F2α injection, blood was drawn for Day 28 PV BVDV SN testing. University of Wyoming Animal Science staff observed animals for estrus and bred them by artificial insemination (AI). In both Trials A and B, insemination of animals began two days after prostaglandin F2α injections (30 days PV). Those not conceiving with the first breeding were bred by AI again after exhibition of a second estrus. After subsequent pregnancy check, those still not pregnant were resynchronized with vaginal implants. Thus each trial had two sets of breeding dates and Cesarean section (C-section) dates.

Blood Sample Collection

Whole blood was drawn for collection of serum to be tested for BVDV SN antibody (by CSU-VDL, Fort Collins, CO) on Day 0 PV (the day of vaccination), Day 28 PV, Day 0 postchallenge (PC), Day 21 PC, Day 35 PC, and just prior to C-section.

Challenge

Challenge was scheduled, based on breeding dates, to occur between days 75 and 85 of gestation. The Trial A and Trial B study animals were challenged by intranasal inoculation with a target challenge dose of NCP BVDV2 or BVDV1b, respectively. The inoculum was prepared and the $TCID_{50}$ was determined prior to challenge. The challenge dose inoculated in Trial A was 4.65 to 4.73 log10 $TCID_{50}$ of virus, and in Trial B the inoculated challenge dose was 4.7 to 5.8 log10 $TCID_{50}$ of virus. A nasal canula was placed on each 3 mL syringe containing 2 mL of inoculum. The contents of the syringe were instilled into a single nostril of each challenged animal.

Clinical Exams, Scoring, and C-section Post-challenge

Body temperatures were taken daily and all animals were clinically scored (Table 1) on the day of challenge (Day 0 PC) and daily thereafter for 21 days PC. We C-sectioned the animals to remove each fetus and treated each dam appropriately for any health complication.

Fetal Tissue Collection and Testing

We recovered fetuses and allantoic and/or amniotic fluids by C-section, 73 to 80 days PC. We necropsied fetuses, noted gross lesions and collected duplicate samples of fetal blood, placenta, liver, spleen, thymus, kidney, heart, lung and brain from each fetus. Tissues from each individual fetus were pooled and placed into a plastic vial and frozen at –94°F (–70°C) for virus isolation. Brain, eyes and remaining tissues were placed into 10% buffered formalin and embedded in paraffin for immunohistochemistry.

Testing Methods

Pooled fetal tissues from each individual fetus and its respective allantoic and/or amniotic fluids were cultured for viral isolation (VI) of BVDV. Immunohistochemistry (IHC) techniques using monoclonal antibody were used to detect BVDV antigen in individual paraffin-embedded tissue sections.[10]

Laboratory personnel followed manufacturer's instructions when using a BVDV antigen enzyme-linked immunosorbent assay (ELISA) test kit[12] to detect BVDV antigen in skin biopsies. They placed the biopsies in test tubes containing 2 mL of PBS and incubated the tubes overnight at 39°F (4°C). One hundred microliter samples were inoculated on anti-BVDV antibody-coated 96-well plates, which were incubated at room temperature for one hour and washed three times using the kit's washing solution. For secondary detection, horseradish peroxidase-conjugated antibody was added to each of the wells, which were incubated. A plate reader and proprietary software were used to analyze results.

Serum Neutralization Antibody Assays

SN antibody titers to BVDV1a and BVDV2 were determined using a microtiter SN format.[11] Sera were complement deactivated by heating at 133°F (56°C) for 30 minutes, and two-fold serial dilutions of each serum were made in triplicate in 96-well microtiter plates. One hundred $TCID_{50}$ of CP BVDV1a (Singer strain) or BVDV2 (125c) was added to duplicate columns of wells, and the serum-virus mixtures were incubated at 98.6°F (37°C) for one hour. The third column of diluted serum served as the serum control. BT cells suspended in MEM were added to each well and the plates were incubated at 98.6°F (37°C) in 5% CO_2 for an additional 72 hours. BT cells were examined for cytopathic effects of the test virus using an inverted light microscope. The presence of SN antibody was indicated by the absence of cytopathic effect (CPE) caused by the BVDV. The SN antibody titer was determined to be the highest dilution of serum that inhibited CPE.

Virus Isolation – Fetal Tissues and Fluids

Pooled fetal tissues from each fetus were used to inoculate confluent monolayers of BT cells (previously tested and shown to be negative for adventitial BVDV infection) in 24 well plates. Virus isolation of fetal tissues was conducted at CSU-VDL for Trial A and at both CSU-VDL and WSVL for Trial B. Virus isolation of fetal fluids was conducted at CSU-VDL for both trials. Lab

Table 1. Clinical Scoring System.

Category	Score	Description
Depression	0	Normal/alert
	1	Slightly depressed/subdued, but easily roused
	2	Moderately depressed/some difficulty in rousing
	3	Severely depressed/somnolent/cannot be roused
Appetite	0	Normal appetite/at the bunk when feed present
	1	Hangs back from feed bunk/picks at hay
	2	Occasionally approaches feed bunk
	3	Not eating/no interest in feed
Respiratory	0	Normal respiratory rate and effort/quiet respirations
	1	Mild increase in respiratory rate and effort/occasional coughing/increased nasal discharge
	2	Moderately dyspneic/increased respiratory rate and effort/coughing/profuse nasal discharge
	3	Severely dyspneic/forced inspiration and expiration/profuse nasal discharge
Gastrointestinal	0	Normal formed feces/if nervous – increased fluidity
	1	Increased fluidity not related to nervous behavior
	2	Watery, mucoid diarrhea/profuse diarrhea/straining to defecate
	3	Blood, mucus, and sloughed tissue in feces/straining to defecate

personnel incubated inoculated BT cells for one hour at 98.6°F (37°C) in 5% CO_2. They removed each inoculum and added 0.5 mL of medium with 2% horse serum, 100 U/mL penicillin, and 100 µg/mL dihydrostreptomycin to each well. Positive (NCP NY-1 BVDV) and negative (media only) control wells were included on each plate. After a four-day incubation, personnel removed the media and used it to inoculate a second 24-well plate of BT cells, which was incubated for an additional seven days. The BT cells in the 24-well plates were air-dried for one hour and then the cells were fixed with 20% acetone: 80% PBS for five minutes; the fixative was decanted; and the plates were allowed to air-dry overnight. Murine monoclonal antibody, biotinylated rabbit anti-mouse IgG and streptavidin-horseradish peroxidase conjugate were used to detect BVDV antigen.

Outcome Definitions

The primary outcome was prevention of infection with BVDV2 and BVDV1b in Trial A and B, respectively. A fetus was considered BVDV-positive if virus was isolated from the pooled tissues or if BVDV was detected by IHC. A fetus was considered BVDV-negative if both VI and IHC were negative. Also, if amniotic or allantoic (fetal) fluids were positive for BVDV, then the fetus was considered infected. Secondary outcome was prevention of clinical disease in the cows. A clinical scoring system quantified reporting of depression, appetite, respiratory signs and gastrointestinal signs as indicated in Table 1. In addition to evaluation of clinical score, fever, pulse and respiratory rates, the appearance of other clinical signs was used to determine presence of clinical disease.

Statistical Analysis

The primary outcome in this study was evaluated by testing the null hypothesis that there was no difference when the infection rate of fetuses from vaccinated animals was compared with the infection rate of fetuses from non-vaccinated control animals by Fisher's Exact test stratified on challenge date. The prevented fraction and 95% confidence intervals were constructed for fetal infection.

We evaluated the secondary outcome for prevention of clinical disease by assessing fever, pulse, respiratory rates and clinical signs. The number of animals with gastrointestinal signs during the observation period was compared between the vaccinated group and the control group by Fisher's Exact test stratified on challenge date. The frequency of gastrointestinal signs was compared between groups by Wilcoxon Rank Sum test using PROC NPAR1WAY stratified on challenge date.

Fever, pulse and respiratory rate were compared between groups in a repeated measure analysis of variance (ANOVA) model with fever, pulse or respiratory rate as the dependent variable and treatment, challenge date, time and the interaction between treatment and time included as independent variables. The best-fit covariance structure was modeled as heterogeneous compound symmetry for fever and pulse, and first order factor analytic for respiratory rate. Baseline rectal temperature, pulse, and respiratory rate were included as covariates in the models for fever, pulse and respiratory rate, respectively.

Since group assignments were made randomly and blindly, any systematic information or measurement bias should be minimal and would expectedly be nondifferential misclassification bias, which would cause a bias toward "no effect" or "no association".

All statistical analyses were performed using the SAS system.[13] The prevented fraction for fetal infection was calculated using Epi Info.[14] The level of significance was set at $P<0.05$.

Results

Primary Outcome

In Trial A, we determined that all 25 vaccinated cows completing the study were carrying normal fetuses with no evidence of *in utero* BVDV infection and that all 10 of the control cows were carrying fetuses PI with BVDV2. In Trial B, we determined that 23/24 vaccinated cows with fetuses at the end of the study had no evidence of *in utero* BVDV infection, and that all six of the control cows with fetuses at the end of the study had evidence of *in utero* BVDV infection as a result of the heterologous BVDV1b challenge. These cows were carrying fetuses PI with BVDV1. The difference in fetal infection rates between vaccinate and control groups was significant ($P<0.05$) in both trials. Fetal protection was 100% for BVDV2 challenge and 96% for BVDV1b challenge.

As stated previously, each trial had two sets of breeding dates and C-section dates because those not conceiving with the first breeding were bred by AI again after exhibition of a second estrus. Thirty-five dams completed Trial A, 10 controls and 25 vaccinates, all of which had fetuses at C-section. The control group contained three mature cows and seven heifers; the vaccinates consisted of five mature cows and 20 heifers. In Trial A, nine controls and 16 vaccinates conceived after the first breeding and one control and nine vaccinates conceived after the second breeding. All of Trial A dams that had a positive pregnancy check after breeding had a fetus at the time of the C-section, thus there were no abortions.

Thirty-three dams completed Trial B, eight controls and 25 vaccinates, but only six controls and 24 vaccinates had fetuses at C-section. In Trial B, eight controls and 20 vaccinates conceived after the first breeding and five vaccinates conceived after the second breed-

ing, but no controls. Two control cows in Trial B had a positive pregnancy check after the first breeding, but did not have a fetus at the time of the C-section. There was also one vaccinate without a fetus at the time of the C-section, although the cow had a positive pregnancy check after the second breeding; thus, there were three abortions in Trial B, one in the vaccinate group and two in the control group.

Clinical Signs

No differences were noted in body temperatures or clinical signs between control and vaccinate groups in Trial A or B.

Fetal age in both trials at challenge ranged from 75 to 97 days of age. In Trial A, the fetal age at challenge ranged from 77 to 80 days for the first set of dams bred and from 75 to 83 days for the second set of dams bred. In Trial B, the fetal age at challenge ranged from 78 to 81 days for the first set of dams bred and from 75 to 97 days for the second set of dams bred.

Fetal age in both trials at time of C-section ranged from 148 to 174 days of age, and fetuses had crown-rump lengths from 14.3 to 22.0 inches (36.2 to 55.9 cm). In Trial A, fetal age at C-section ranged from 158 to 160 days for the first set of dams bred and from 148 to 156 days for the second set of dams bred. In Trial B, fetal age at C-section ranged from 151 to 155 days for the first set of dams bred and from 152 to 174 days for the second set of dams bred.

Serological Analysis Postvaccination

In both trials, the geometric mean titer (GMT) to BVDV1 and BVDV2 remained at <2 for the control groups from the time of vaccination to the time of challenge. In contrast, in the vaccinated group the BVDV1 GMT rose moderately (to >1351 and 867 in Trials A and B, respectively) and the BVDV2 GMT rose mildly (to 36 and 34 in Trials A and B, respectively) before challenge (Tables 2 and 3).

Serological Analysis Post-challenge

The starting GMT for control groups (Day 0 PC) in both Trial A and B was <1:2. After challenge with BVDV2 (Trial A) the GMT for BVDV2 rose dramatically for controls (>2048), as indicated in Table 4. The vaccinates in Trial A started out (Day 0 PC) with a BVDV2 GMT of 36, which rose to 388 by Day 72 PC. The Day 0 PC BVDV1 GMT was >1351, which changed to 2288 by Day 21 PC and remained fairly stable for the rest of the study. The control group BVDV1 antibody response to BVDV2 challenge was a steady GMT increase to 21 by the end of the study.

The GMT of the groups in Trial B (BVDV1b challenge – see Table 5) was highest for BVDV1 in the vac-

Table 2. Trial A - Serum neutralization GMT[α] postvaccination.

SN group	Animal Group	Day 0 PV	Day 28 PV	Day 110-128 PV[β]
BVDV2	Control	<2[δ]	<2	<2
	Vaccinate	<2	9	36
BVDV1	Control	ND[ε]	<2	<2
	Vaccinate	ND	236	>1351

[α] Geometric Mean Titer
[β] Same as day of challenge (Day 0 PC); Day 110 for 25/35 cows, Day 128 for 10/35 cows
[δ] Titers <2 were translated to 1 to calculate GMT
[ε] ND=Not Done

Table 3. Trial B - Serum neutralization GMT[α] postvaccination.

SN group	Animal Group	Day 0 PV	Day 28 PV	Day 114-150 PV[β]
BVDV2	Control	ND[ε]	<2[δ]	<2
	Vaccinate	ND	3	34
BVDV1	Control	<2	<2	<2
	Vaccinate	<2	160	867

[α] Geometric Mean Titer
[β] Same as day of challenge (Day 0 PC); Day 114 PV for 28/33 cows, Day 150 PV for 5/33 cows
[δ] Titers <2 were translated to 1 to calculate GMT
[ε] ND=Not Done

cinate group on Day 35 PC (1144). This vaccinate group also had a numerically high BVDV1 GMT on Day 0 PC (867) compared with BVDV2 GMT (34). The vaccinate group BVDV2 GMT did not change a great deal numerically during the study. The control group GMT for both BVDV2 and BVDV1 rose steadily, topping out at 19 and 38, respectively, on Day 72 PC.

Fetal Tissue Viral Isolation and PCR

As stated previously, VI of fetal tissues was conducted at CSU-VDL for Trial A and at both CSU-VDL and WSVL for Trial B. In Trial A, BVD virus was isolated from 10 fetuses, all from the unvaccinated control group. Fetal tissues from all the fetuses were tested at CSU-VDL for BVDV genotype using PCR. Ten fetuses were positive for genotype 2; all 10 were from control cows.

In Trial B, CSU-VDL isolated BVDV from seven fetuses – six isolations were from control fetuses and one isolate was from a vaccinated dam's fetus. Following the results from CSU-VDL, the duplicate sets of individual pooled tissues were cultured for virus at the WSVL, and the VI-positives were tested for genotype using PCR. WSVL isolated BVDV from six fetuses, all from the six control cows. WSVL's PCR tests determined that each of the six isolates were genotype 1. The reason CSU-VDL detected virus from a vaccinate's fetus and WSVL didn't isolate BVDV from any fetus from a vaccinate could not be determined. The thawed tissues were not in adequate condition for further testing.

Fetal Fluids Viral Isolation, IHC, and Skin Biopsy ELISA for BVDV

As previously stated, VI of fetal fluids was conducted at CSU-VDL for both trials. In Trial A, the results of VI of fetal fluids, IHC and skin biopsy ELISA coincided with results of VI of fetal tissues, with the exception that in three control VI fetal fluid samples tested negative. In Trial B, all six control fetuses identified at C-section tested positive for IHC and skin biopsy ELISA, and four of six tested positive on VI of fetal fluids. The vaccinate group in Trial B all tested negative for VI of fetal fluids, IHC and skin biopsy ELISA with the exception of one fetus that was IHC positive—the same fetus that had positive VI of fetal tissue.

Discussion

The degree of antigenic drift associated with RNA Pestiviruses, and the resultant variation in viral virulence and infectivity, resulted in the concern that monovalent vaccines would not adequately control various strains of BVDV.[6] Previous trials have shown significant variation in the ability of BVDV1a vaccines to prevent fetal infection when challenged by BVDV2 viruses.[2,18] However, a recent study using a monovalent NCP BVDV1a vaccine prior to breeding demonstrated 93% fetal protection in dams challenged with BVDV2 (PA131 strain).[3]

In Trial A, 100% of fetuses tested negative follow-

Table 4. Trial A - Serum neutralization GMT$^{\alpha}$ postchallenge with BVDV2.

SN group	Animal Group	Day 0 PC	Day 21 PC	Day 35 PC	Day 72 PC
BVDV2	Control	<2$^{\delta}$	104	≥588	>2048
	Vaccinate	36	367	286	388
BVDV1	Control	<2	2	17	21
	Vaccinate	>1351	2288	2353	2048

$^{\alpha}$ Geometric Mean Titer
$^{\delta}$ Titers <2 were translated to 1 to calculate GMT

Table 5. Trial B - Serum neutralization GMT$^{\alpha}$ postchallenge with BVDV1b.

SN group	Animal Group	Day 0 PC	Day 21 PC	Day 35 PC	Day 72 PC
BVDV2	Control	<2$^{\delta}$	1	3	19
	Vaccinate	34	43	25	23
BVDV1	Control	<2	2	9	38
	Vaccinate	867	996	1144	471

$^{\alpha}$ Geometric Mean Titer
$^{\delta}$ Titers <2 were translated to 1 to calculate GMT

ing challenge with a BVDV2 isolate that infected 100% of the fetuses from non-vaccinated dams.

In Trial B, the prevented fraction (prevented fraction $1 - p_2/p_1$, where p_2 are animals with BVDV-positive fetuses in the vaccinated group and p_1 are the positive fetuses in the control group) in vaccinated cows with fetuses was 96%. Three animals, one vaccinate and two controls, were not pregnant at the time of C-section. The pens containing the cows were checked daily, but no aborted fetuses were observed. The reason for not finding fetuses could be due to predators. Coyotes were often seen near the pens and could have easily removed any aborted fetus. Also, the fetuses could have been reabsorbed by the dams. The loss of these fetuses could have been due to the BVDV challenge or to other causes. The prevented fraction in vaccinated cows including those that aborted, and assuming the abortions were due to BVDV, would be 92%.

The isolates identified by Fulton *et al*[7,8] were 45.8% BVDV1b, 28.2% BVDV1a and 26% BVDV2a, and the genotypes found in PI cattle entering a Kansas feedlot were 77.9% BVDV1b, 11.6% BVDV1a and 10.5% BVDV2a. Efforts to control BVD should include testing and identifying PI calves at a young age so that exposure to other animals is minimized. In addition, it is imperative to vaccinate cows prior to breeding using BVDV vaccines that have been demonstrated to be effective against fetal infection with common genotypes.

Conclusion

In these studies, vaccination with a monovalent BVDV1a vaccine resulted in 96% fetal protection against BVDV1b challenge and 100% fetal protection against BVDV2 challenge. Results of these studies support label claims that the vaccine will aid in the prevention of persistent BVDV1 and BVDV2 infection of the fetus when used as directed in the cow or heifer 30 to 60 days before breeding.

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Endnotes

[1] Pyramid® 9, Fort Dodge Animal Health, Fort Dodge, IA

[2] Sigma Chemical Co., LTD., St. Louis, MO

[3] Opti-MEM®, GibcoTM Invitrogen Corporation, Carlsbad, CA

[4] Provided by E. J. Dubovi, Cornell University, Ithaca, NY

[5] Zymed Laboratories, So. San Francisco, CA

[6] Sigma Chemical Co., LTD., St. Louis, MO

[7] Gibco, So. San Francisco, CA

[8] Fisher Scientific, Pittsburgh, PA

[9] MGA 500 Liquid Premix, Pharmacia, Kalamazoo, MI

[10] EAZI-BREED CIDR, Pharmacia, Kalamazoo, MI

[11] Lutalyse, Pharmacia, Kalamazoo, MI

[12] Bovine Viral Diarrhea Virus Antigen Test Kit, Syracuse Bioanalytical Inc., Ithaca, NY

[13] SAS Version 8, SAS Institute, Inc., Cary, NC

[14] Epi Info™, Centers for Disease Control and Prevention, Atlanta, GA

References

1. Baker JC: The clinical manifestations of bovine viral diarrhea infection. *Vet Clin North Am Food Anim Pract* 11:425-445, 1995.

2. Brock KV, Cortese VS: Experimental fetal challenge using Type II bovine viral diarrhea virus in cattle vaccinated with modified-live virus vaccine. *Vet Ther* 2:354-360, 2001.

3. Brock KV, McCarty K, Chase CC, *et al*: Protection against fetal infection with either bovine viral diarrhea virus type 1 or type 2 using a noncytopathic type 1 modified-live virus vaccine. *Vet Ther* 7:27-34, 2006.

4. Carruthers TD, Petrie L: A survey of vaccination practices against bovine viral diarrhea (BVD) virus in Saskatchewan dairy herds. *Can Vet J* 37:621-622, 1996.

5. Cortese VS, Grooms DL, Ellis J, *et al*: Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhea virus type 1 by use of a modified-live virus vaccine. *Am J Vet Res* 59:1409-1413, 1998.

6. Fairbanks KK, Rinehart CL, Ohnesorge WC, *et al*: Evaluation of fetal protection against experimental infection with type 1 and type 2 bovine viral diarrhea virus after vaccination of the dam with a bivalent modified-live virus vaccine. *J Am Vet Med Assoc* 225:1898-1904, 2004.

7. Fulton RW, Hessman B, Johnson BJ, *et al*: Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *J Am Vet Med Assoc* 228:578-584, 2006.

8. Fulton RW, Ridpath JF, Ore S, *et al*: Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. *Vet Microbiol* 111:35-40, 2005.

9. Goens SD: The evolution of bovine viral diarrhea: a review. *Can Vet J* 43:946-954, 2002.

10. Haines DM, Clark EG, Dubovi EJ: Monoclonal antibody-based immunohistochemical detection of bovine viral diarrhea virus in formalin-fixed, paraffin-embedded tissues. *Vet Pathol* 29:27-32, 1992.

11. Malmquist W: Bovine viral diarrheal-mucosal disease: etiology, pathogenesis and applied immunity. *J Am Vet Med Assoc* 156:763-768, 1968.

12. McClurkin AW, Littledike ET, Cutlip RC, *et al*: Production of cattle immunotolerant to bovine viral diarrhea virus. *Can J Comp Med* 48:156-161, 1984.

13. Pailsey L, Wells S: Peracute bovine viral diarrhea reportedly spreading. *J Am Vet Med Assoc* 205:391-392, 1994.

14. Pellerin C, van den Hurk J, Lecomte J, *et al*: Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. *Virology* 203:260-268, 1994.
15. Reed L, Muench H: A simple method of estimating fifty per cent endpoints. *Am J Hygiene* 27:493-497, 1938.
16. Ridpath JF, Bolin SR, Dubovi EJ: Segregation of bovine viral diarrhea virus into genotypes. *Virology* 205:66-74, 1994.
17. Saliki JT, Fulton RW, Hull SR, *et al*: Microtiter virus isolation and enzyme immunoassays for detection of bovine viral diarrhea virus in cattle serum. *J Clin Microbiol* 35:803-807, 1997.
18. Van Campen H, Vorpahl P, Huzurbazar S, *et al*: A case report: evidence for type 2 bovine viral diarrhea virus (BVDV)-associated disease in beef herds vaccinated with a modified-live type 1 BVDV vaccine. *J Vet Diagn Invest* 12:263-265, 2000.

Abstract

Proteomic Evaluation of Tissues at Functionally Important Sites in the Bovine Claw

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The endemic nature of lameness arising from horn disorder in dairy cows has focused attention on the biology of the bovine claw. The production of lesions causing lameness has been associated with impairments of horn production in the epidermis and connective tissue turnover in the dermis in functionally important regions of the claw.

This paper provides an overview of recent studies which investigated the application of contemporary methodology to the determination of protein expression in the tissues of the epidermis and dermis. It provides data derived from a proteomics approach based on 2-dimensional gel electrophoresis of extracts of tissues obtained from different functional sites of the claw. The procedure used separates proteins according to differences in charge and molecular mass with examination of gels effected by application of analytical software.

Images of gels were obtained for extracts of cornified horn and soft (uncornified) tissue and soft tissue explants obtained from coronary, laminar, solear and heel regions of claws of beef and dairy cattle. Commonalty and certain differences were apparent in profiles of protein spots representative of these anatomical regions particularly in the ranges typical of keratins (40-70kDa) and those of lower molecular mass (<40kDa). Initial identification of certain protein "spots" by immunoblotting and peptide mass fingerprinting on gels indicated the presence of cytoskeletal and hard keratins, heat shock proteins and proteins involved in energy metabolism and transport of trace minerals and fatty acids. Data were also presented from an immunohistochemical examination of sections of claw tissues for certain intermediate filament and microfilament proteins. The results highlight the additional importance of defining precise cellular or extracellular location of proteins within the tissue. It is concluded that further work will be needed to characterise the individual proteins identified from the protein profiles so that (i) relationships with known differences in amino acid composition and (ii) precise roles in supporting physical function in healthy and pathological states, may be better understood. Attention is also drawn to investigative methodology based on protein identification as a valuable means of advancing research investigating the biology of the bovine claw.