

# Response of calves persistently infected with noncytopathic bovine viral diarrhoea virus (BVDV) subtype 1b after vaccination with heterologous BVDV strains in modified live virus vaccines and *Mannheimia haemolytica* bacterin-toxoid

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## Abstract

Seronegative persistently infected (PI) calves with bovine viral diarrhoea virus (BVDV) subtype 1b were vaccinated with each of four modified live virus (MLV) BVDV vaccines and a *Mannheimia haemolytica* bacterin-toxoid. Nasal swabs and peripheral blood leukocytes (PBL) were collected for virus isolation and serums were collected after vaccination and tested for BVDV1a, BVDV1b, BVDV2, bovine herpesvirus-1 (BHV-1), bovine parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV) antibodies. *M. haemolytica* and *Pasteurella multocida* antibodies were detected using ELISA procedures. None of the PI calves developed mucosal disease (MD) after MLV vaccination. None of the BVDV PI calves seroconverted to BVDV1b after MLV vaccination. Calves receiving MLV vaccines seroconverted to the respective type/subtype in the vaccine. Calves receiving a MLV vaccine with noncytopathic (NCP) BVDV1 (subtype not designated) did not seroconvert to BVDV1a, BVDV1b, or BVDV2. The PI calves were positive for BVDV subtype 1b, in the PBL and nasal swabs throughout the study. Calves receiving each of three vaccines with known BVDV1a strains had BVDV1a positive samples after vaccination, in some but not all calves, up to Day 28. The PI BVDV1b calves did not respond with increased *M. haemolytica* antibodies after vaccination compared to BVDV negative calves receiving the same *M. haemolytica* vaccine.

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## 1. Introduction

Bovine viral diarrhoea virus (BVDV) is present in the US and other countries worldwide [1]. BVDV are classified as biotypes based on presence or absence of visible cytopathic effects in infected cell cultures: cytopathic (CP) or noncytopathic (NCP) [1]. There are also genotypic and antigenic differences which separate the BVDV into types 1 and 2 with further differentiation into subtypes 1a and 1b [2–4]. BVDV is responsible for numerous clinical syndromes, including

acute infections, as well as conditions associated with immunosuppressive effects of the viral infection [1]. Fetal infections may occur when the susceptible cows/heifers develop a viremia after the initial acute infection. There are several outcomes of the fetal infections depending on gestational stages when the fetus is exposed. In utero infections of bovine fetuses with NCP BVDV between Days 42 and 125 may cause persistent infections (PI) of the fetus which then remain immunotolerant to the infecting BVDV [5]. The fetuses are carried to term, remain seronegative to the infecting virus, and shed the BVDV throughout their lifetime. The PI calves are considered a principal reservoir to expose susceptible cattle [1].

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A severe clinical form of BVDV called Mucosal disease (MD) occurs in cattle. Calves PI with BVDV develop MD when they are subsequently infected with a related CP BVDV strain [6,7]. Postvaccinal reactions resembling MD occur after calves have received modified live virus (MLV) BVDV vaccines, although the occurrence is low [8–11]. The MLV BVDV vaccines for cattle contain the CP biotype in all but one vaccine [12]. This has led to speculation/inference that PI calves receiving MLV BVDV vaccines develop MD.

The objective of the study was to determine the host response of PI infected calves with NCP BVDV1b after vaccination with MLV vaccines with various BVDV types and subtypes, and to determine whether PI calves could develop serum antibodies to a bacterial immunogen, *Mannheimia haemolytica* bacterin-toxoid.

## 2. Materials and methods

### 2.1. Calves

Eight calves were obtained from a ranch undergoing reproductive loss in the breeding herd. The calves born between 24 March 2001 and 9 April 2001 were identified as PI based on BVDV positive immunohistochemistry testing us-

ing ear notch samples [13]. The calves were then delivered to the facilities at Oklahoma State University where they were isolated from other cattle. The pen contained an individual waterer, and the calves received hay and grain mixture feed.

### 2.2. Sample collection

The EDTA containing blood tubes for peripheral blood leukocytes (PBL) preparation and nasal swabs for viral isolation were collected. Serums were also prepared for antibody testing and viral isolation.

### 2.3. Virus isolation and serologic tests

Virus neutralization tests using Madin–Darby bovine kidney (MDBK) cells in 96-well microtiter plates were used to quantitate viral neutralization antibodies to BVDV using the CP Singer strain for 1a; TGAC CP strain for 1b; 125 CP strain for type 2; parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV) as previously described [12,14–18]. A plaque reduction assay in 24-well plates with MDBK monolayers was used to quantitate bovine herpes virus 1 (BHV-1) antibodies [14,16,18]. Antibodies to *M. haemolytica* whole cell, *M. haemolytica* leukotoxin, and *Pasteurella multocida* outer membrane protein (OMP) were assayed by ELISA tests [18].

Table 1  
Viral neutralizing serum antibody titers to BVDV1a, BVDV1b, BVDV2, BHV-1, and BRSV in calves receiving MLV vaccines<sup>a</sup>

Virus	Days	Vaccine 1		Vaccine 2		Vaccine 3		Vaccine 4	
		A <sup>b</sup>	B	C	D	E	F	G	H
BVDV1a	0	0 <sup>c</sup>	0	0	0	0	0	0	0
	14	0	0	8	64	0	0	8	4
	28	64	64	128	1024	0	0	16	No sample
	42	64	64	1024	8192	0	0	64	No sample
BVDV1b	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0	No sample
	35	0	0	0	0	0	0	0	No sample
	42	0	0	0	0	0	0	0	No sample
BVDV2	0	0	0	0	0	0	0	0	0
	14	32	32	0	0	0	0	0	0
	28	1024	512	0	0	0	0	0	No sample
	42	1024	2048	0	0	0	0	0	No sample
BHV-1	0	0	16	0	0	0	0	0	0
	14	24	12	0	0	149	116	50	34
	28	58	14	22	0	109	65	62	No sample
	42	59	0	15	0	65	73	49	No sample
BRSV	0	8	8	4	0	0	4	4	4
	14	8	32	64	4	16	32	8	8
	28	8	16	64	4	8	16	4	No sample
	42	4	32	16	4	8	8	4	No sample

<sup>a</sup> Vaccine 1 contained BVDV1a C24V and BVDV2 125C; Vaccine 2 contained BVDV1a Singer; Vaccine 3 contained BVDV1 WRL (nondesignated subtype); and Vaccine 4 contained BVDV1a NADL.

<sup>b</sup> Calves A and B received Vaccine 1; C and D, Vaccine 2; E and F, Vaccine 3; and G and H, Vaccine 4.

<sup>c</sup> No neutralization at 1:4, the lowest dilution tested for BVDV1a, BVDV1b, BVDV2, PI-3V, BRSV or no neutralization at 1:10, the lowest dilution tested for BHV-1.

Virus isolation from PBL, nasal swabs, and serums were attempted as described using either MDBK or bovine turbinate (BT) cells as substrates [17]. The BVDV isolates were subtyped as 1a, 1b, or 2 by differential polymerase chain reaction (PCR) testing, and the 5'-UTR region was sequenced for selected isolates [4].

#### 2.4. Vaccines

The MLV vaccines used in the study contained BHV-1, BVDV, PI-3, and BRSV. The vaccines and their respective BVDV biotype (CP or NCP) and subgenotype are: Vaccine 1,<sup>1</sup> CP BVDV1a C24V strain and CP BVDV2, 296 strain; Vaccine 2,<sup>2</sup> CP BVDV1a Singer; Vaccine 3,<sup>3</sup> NCP BVDV1 (subtype not identified) WRL strain; and Vaccine 4,<sup>4</sup> CP BVDV1a NADL strain.

The bacterial vaccine contained *M. haemolytica* bacterin-toxoid.<sup>5</sup>

#### 2.5. Experimental design of vaccine study

On 29 June 2001, two calves each received one of four MLV vaccines (Table 1) according to the label instructions. The calves were all seronegative to BVDV1a, BVDV1b, and BVDV2 on Day 0. Selected calves may have had maternal antibodies to BHV-1, and/or BRSV on Day 0 (Table 1).

The PI calves each received the *M. haemolytica* bacterin-toxoid on Day 0 by the subcutaneous route. A control group of eight healthy BVDV-free calves, housed at another facility received the same vaccine. Four healthy BVDV-free calves served as nonvaccinate controls. Samples including PBL and nasal swabs were obtained from the MLV vaccinated calves on Days 0, 4, 7, 10, 14, 21 and 28 for viral isolation. Nasal swabs for viral isolation were also collected on Days 35 and 42. Serums for antibody testing were prepared on Day 0 and weekly thereafter until Day 56.

### 3. Results

#### 3.1. Clinical observations

None of the PI calves developed clinical signs of MD during the study. One calf (H) that received Vaccine 4 died on Day 18. Pathological examination revealed a severe suppurative and ulcerative enterocolitis. Samples collected prior to death, PBL and nasal swab, as well as an organ pool from necropsy tissues were each positive for NCP BVDV. No CP strains suggesting vaccine origin or MD related virus were isolated.

#### 3.2. Virus isolations

The PBL and nasal swabs in the eight PI calves were all positive for NCP BVDV at Day 0 (prior to vaccination). The PBL were positive for BVDV throughout the study to Day 28. The isolates from the PBL subsequent to MLV vaccination remained NCP with a few exceptions. Calf D that received Vaccine 2 containing a CP strain had a CP strain isolated from the PBL on Day 4. Calf B that received Vaccine 1 containing two different CP strains had a CP strain isolated from the PBL on Day 7. Calves A and B that received Vaccine 1 containing two different CP strains had a CP strain isolated from the PBL on Day 14. Neither calves that received the Vaccine 3 containing NCP BVDV had any CP BVDV strains in the PBL after vaccination. All of the nasal swab samples were positive from postvaccination Days 0–42, and the BVDV isolated were all NCP on Days 0, 28, 35, and 42. Cytopathic strains were found in the nasal swabs of several calves between Days 4 and 21, although the appearance of the CP was equivocal.

The presence of BVDV1a strains in the PBL and nasal swabs are indicated in Table 2. No BVDV1a was detected in Day 0 samples. BVDV1a was isolated from 5/6 calves receiving MLV vaccines with positives in the PBL and/or nasal swabs between Days 7 and 28. None of the samples from the two calves receiving NCP BVDV1 were positive for BVDV1a. It is likely there were NCP BVDV1b positives along with the CP isolates. Attempts have not been made to separate or clone the CP and NCP BVDV positive samples.

#### 3.3. Antibody titers

The antibody titers to the viruses are listed in Table 1. The vaccinated calves did not develop any detectable BVDV1b antibody titers after vaccinations. The calves responded with BVDV antibodies to the respective BVDV subtype in the vaccine. The only exception was calf H receiving Vaccine 4 which died on Day 18. It had only low antibody tiers (4) to BVDV1a and BVDV1b. Titers of 4 were not significant as there is not a four-fold or greater increase from 0 titer at Day 0 ( $0 < 4$ ). Calves A and B responded with both BVDV1a and BVDV2 antibodies as expected as the Vaccine 1 contained both BVDV1a and BVDV2 strains. Calves C and D responded with only BVDV1a antibodies as the Vaccine 2 contained only BVDV1a. Calves E and F receiving Vaccine 3 containing NCP BVDV1 (undesignated) did not develop antibodies to BVDV1a, BVDV1b, or BVDV2. The one calf surviving throughout the study, and receiving Vaccine 4 containing BVDV1a, developed BVDV1a antibodies after vaccination.

The MLV vaccines stimulated BHV-1 antibodies (two-fold or greater) after Day 0 in most calves. One calf, B had maternal BHV-1 antibodies and did not respond with increased BHV-1 antibodies; and calf D remained seronegative after vaccination. Four of eight calves responded with increased BRSV antibodies (four-fold or greater). Low

<sup>1</sup> Titanium<sup>TM</sup>5, Agri Laboratories, St. Joseph, MO 64503.

<sup>2</sup> Pyramid<sup>®</sup> MLV 4, Fort Dodge Animal health, Fort Dodge, IA 50501.

<sup>3</sup> Jencine<sup>®</sup>4, Schering Plough Animal Health, Anion, NJ 07083.

<sup>4</sup> Bovi-Shield<sup>TM</sup>4, Pfizer, Inc., Animal Health Group, Exton, PA 19341.

<sup>5</sup> One Shot<sup>®</sup>, Pfizer, Inc., Animal Health Group, Exton, PA 19341.

Table 2  
Presence of BVDV1a in virus positive samples from PI BVDV1b calves after MLV vaccination

Calf no.	Vaccine	Day (PBL/nasal)								
		0	4	7	10	14	21	28	35	42
A	1	- <sup>a</sup> /-	-/-	-/+ <sup>b</sup>	-/+	+/+	-/+	-/-	NS <sup>c</sup> /-	NS/-
B	1	-/-	-/-	-/+	+/+	+/+	-/-	-/-	NS/-	NS/-
C	2	-/-	-/-	-/-	-/+	-/-	-/+	-/-	NS/-	NS/-
D	2	-/-	+/+	+/+	-/+	-/-	-/-	+/-	NS/-	NS/-
E	3	-/-	-/-	-/-	-/-	-/-	-/-	-/-	NS/-	NS/-
F	3	-/-	-/-	-/-	-/-	-/-	-/-	-/-	NS/-	NS/-
G	4	-/-	-/-	+/+	+/+	-/-	-/+	-/-	NS/-	NS/-
H	4	-/-	-/-	-/-	-/-	-/-	NS/NS	NS/NS	NS/NS	NS/NS

<sup>a</sup> (-): no BVDV1a detected.

<sup>b</sup> (+): positive for BVDV1a.

<sup>c</sup> NS: no sample available.

Table 3  
Antibody titers to *M. haemolytica* and *P. multocida* in BVDV1b PI calves receiving *M. haemolytica* bacterin-toxoid: increase in titers over baseline at Day 0

Antigen	Group	Day postvaccination (mean ratio) <sup>a</sup>			
		14	28	42	56
<i>M. haemolytica</i> , leukotoxin	Controls	1.07 a	1.28 a	1.36 a	1.34 a
	PI vaccinates	1.01 a	1.58 a	1.60 a	1.64 a
	Healthy vaccinates	24.6 b	15.77 b	13.77 a	9.59 a
<i>M. haemolytica</i> , whole cell	Controls	1.13 a	0.99 a	1.12 a	1.42 a
	PI vaccinates	1.91 a	4.85 a	4.90 a	3.18 a
	Healthy vaccinates	15.93 b	10.86 b	7.24 a	4.92 a
<i>P. multocida</i> , OMP	Controls	1.2 a	1.29 a	0.98 a	0.89 a
	PI vaccinates	1.06 a	1.13 a	1.28 a	1.36 a
	Healthy vaccinates	1.13 a	1.19 a	1.25 a	1.19 a

Mean ratio of value of the particular day/baseline.

<sup>a</sup> Means with the same letter are not significant using  $\alpha = 0.10$ .

levels of antibodies at Day 0, presumably maternal in the other four calves appeared to block active immune response to BRSV vaccination. Calf G, seronegative to PI-3V at Day 0, responded with increased PI-3V antibodies after vaccination. Two calves seronegative to PI-3V at Day 0 failed to develop PI-3V antibodies. Four PI-3V seropositive calves (titer from 16 to 64) had declining antibody titers after vaccination, suggesting a blocked active humoral response to PI-3V vaccination.

The PI calves vaccinated with the *M. haemolytica* bacterin-toxoid had significantly lower antibody responses to *M. haemolytica* whole cell and leukotoxin antibodies than did the vaccinated healthy BVDV-free calves (Table 3). Means are mean ratio of value/baseline. Alpha (0.10) was used to make comparisons due to small sample size and large standard errors. These differences in ratios were significant for Days 14 and 28 for antibodies to *M. haemolytica* leukotoxin and *M. haemolytica* whole cell antigens with the non PI healthy vaccinated calves having significantly higher ratio of antibodies compared to the PI calves and nonvaccinated controls. There was no increase in *P. multocida* antibodies as expected because the vaccine did not contain *P. multocida* immunogens.

#### 4. Discussion

The results of this study support other studies whereby PI BVDV cattle were able to respond to BVDV immunogens other than to the specific BVDV inducing immunotolerance [5,20,21]. The present study is unique in that these above cited studies were performed without the BVDV strain of the PI calves identified as to type/subtype. The studies using genetic differences detected by PCR and sequencing were not reported until the 1990s [2–4]. McClurkin et al. [5] reported that the 7443, NY-1, VM, and MC strains induced PI calves in susceptible fetuses. The subtypes for each of the strains were later subtyped as: 7443, BVDV1b; NY-1, BVDV1b; and VM, BVDV1a. The BVDV subtype for MC the strain is not known. PI calves in that study developed antibodies to other viral and bacterial agents. In that study [5], four PI bulls exposed to BHV-1 and PI-3V developed neutralizing antibodies to each virus ranging from <1:4 to >1:128. None of six PI bulls injected intravenously with their homologous BVDV developed BVDV antibodies. Bolin et al. [20] vaccinated two-year-old steers PI with the BVDV Nebraska strain (later to be determined as 1b), with each of three vaccines: MLV BVDV CP NADL (later to be determined as

1a); MLV BVDV CP Singer (later to be determined as 1a); and a killed vaccine with BVDV Singer. No clinical signs of MD were observed after six weeks of vaccination. However, MD developed when the PI calves were exposed to a CP BVDV strain, TGAC (later to be determined as 1b). Most serums collected at necropsy from the nine PI animals indicated that the PI animals remained seronegative to the PI strain, Nebraska BVDV (later to be determined as 1b). There were calves with titers to the 7443 strain, BVDV (later to be determined as 1a), and calves with titers to the Singer strain, BVDV (later to be determined as 1a). In another study Bolin [21] reported that three cows PI with the Nebraska BVDV (later to be determined as 1b) strain were vaccinated with MLV vaccine containing CP BVDV NADL strain (later to be determined as 1a). The samples collected three weeks postvaccination from the PI animals had antibody titers to BVDV 5960 strain (later to be determined as 1a) and BVDV Singer strain (later to be determined as 1a). The PI BVDV1b calves remained seronegative to the Nebraska, NY-1, and TGAN (later to be determined as 1b). The studies [5,20,21] were performed without knowledge of the BVDV/subtype status for any of the strains: the PI, vaccine, or challenge strains. Current knowledge of the various BVDV subtypes allows interpretation of the results of these prior studies [5,20,21] particularly relating to serum antibody response to heterologous BVDV strains in PI animals.

Houe and Heron [19] reported that PI calves responded with cellular immune responses including cutaneous tuberculin test after Johne's disease immunization. Also the PI calves became sensitized to the skin sensitizing agent, DNCB, and developed serum antitoxin after tetanus immunization. That study also reported that the PI calves response to these antigens was not significantly different compared to non PI calves. Thus, PI calves can respond to other non-BVDV antigens with both cellular and humoral responses. However, in the present study, PI calves failed to immunologically respond to *M. haemolytica* bacterin-toxoid. BVDV is known to cause immunosuppression and PI calves may remain immunotolerant to some antigens, but not others.

The results of the current study confirm that PI calves can respond to heterologous BVDV strains, particularly vaccine strains. The initial attempts to eliminate PI BVDV animals utilized the presence of BVDV antibodies to declare an animal as a non PI animal. This was an erroneous approach to detecting and eliminating PI animals. Review of diagnostic laboratory accessions found PI calves that had BVDV1a serum antibodies. The BVDV isolates were subsequently found to be BVDV2 based on PCR. These calves were later found to have been vaccinated with a MLV vaccine containing BVDV1a NADL strain [24]. In another study, a PI calf with the BVDV1b strain developed BVDV1a serum antibodies to the Singer strain following vaccination with MLV BVDV1a Singer vaccine [25]. Results from these two studies and the current study underscore the point that the presence of BVDV antibodies after loss of maternal antibodies does not necessarily indicate non PI status.

The ability of the MLV BVDV vaccine virus to be shed in PI calves is supported by this study. Nasal swabs and PBL collected postvaccination contained BVDV1a from some, but not all, of the calves receiving BVDV1a containing vaccines. The interval of the BVDV1a detection was between Days 4 and 28. There is the potential that new BVDV strains could develop after PI calves are vaccinated with MLV vaccines containing heterologous BVDV. Ridpath and Bolin [22] reported that a NCP and CP pair of BVDV2 were isolated from an animal dying of MD three months after vaccination with a MLV BVDV1a NADL vaccine. The CP strain, BVDV2-125C, had an insertion in the genome with sequences from the vaccine virus. Thus, MD may result from recombination between the PI virus and an exogenous virus such as a vaccinal strain [22]. The current isolates from this study will be examined for possible genetic recombination.

Bovine respiratory diseases (BRD) including "Shipping Fever" are bacterial pneumonias caused by *M. haemolytica*, *P. multocida*, and occasionally by *Haemophilus somnus*. These bacterial pneumonias are associated with stress and may often be predisposed by viruses such as BHV-1, BVDV1a, BVDV1b, BVDV2, PI-3V, BRSV, bovine coronaviruses, and bovine adenoviruses [12,17]. The PI BVDV animals shedding virus may render the contact cattle more susceptible to bacterial infections [12] as BVDV is immunosuppressive with reduced lymphocyte, neutrophil, and macrophage functions [1]. Pneumonia may also be found in PI BVDV calves at necropsy [23,26]. In a study of PI calves from a single herd moved to a feedlot and observed over time, 25% of the PI calves had gross pneumonic lesions at necropsy, with no or only mild MD lesions [23]. McClurkin et al. [5] also reported that when PI calves were exposed to *M. haemolytica* by aerosol, one of four calves died of acute pasteurellosis within three days, and the other three calves became febrile with eventual recovery and increasing *M. haemolytica* antibody titers. The reduced immune response to the *M. haemolytica* immunogens in this study suggest that the PI calf may be more susceptible to virulent *M. haemolytica* or other bacterial respiratory pathogens.

In conclusion, vaccination of PI calves with heterologous BVDV strains did not induce MD in the 42-day interval of the study. The calves mounted an immune response detected by serum antibodies to the non1b BVDV subtypes in the vaccines. During the study the calves continued to shed virus including heterologous BVDV1a strains; however, the duration of the detectable BVDV1a shedding ceased before the end of the study. The PI calves, while responding to BVDV vaccinal immunogens, did not respond as well as healthy non PI calves to *M. haemolytica* vaccination.

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