Vaccine associated mucosal disease case study: demonstrating the importance of subsequent herd PI testing

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Abstract

Mucosal disease (MD) affected a single-source group of 461 recently purchased yearling steers shortly after vaccination with a multivalent vaccine containing 2 strains of modified-live bovine viral diarrhea virus (BVDV). Terminal disease affected 21 steers at 18 to 66 days post-vaccination, and was confirmed as mucosal disease in 3 carcasses examined postmortem. An additional 17 animals identified as persistently infected remained asymptomatic during the study. A laboratory study was undertaken to determine whether mucosal disease was triggered by a BVDV in the multivalent vaccine. Purified cytopathic BVDV isolates from 2 animals that died early in the outbreak were genetically identical (>99%) to the vaccine type 2 BVDV strain 125a in 3 genomic regions: 5'-untranslated region to the N^{pro}, E2, and NS2/3. This genetic identity combined with the presence of a single peak outbreak occurring soon after vaccination suggests that the vaccine BVDV was the cause of mucosal disease in these steers. The investigation underscores laboratory challenges in determining the role of vaccinal BVDV when mucosal disease affects multiple animals within 2 months of vaccination, and the need for laboratory testing to identify all persistently infected animals.

Key words: bovine viral diarrhea, BVD, mucosal disease, PI, cattle, vaccine

Résumé

La maladie des muqueuses a affligé un groupe de 461 bouvillons de l'année provenant d'une seule source et achetés récemment peu de temps après la vaccination avec un vaccin multivalent contenant deux souches de virus vivants modifiés de la diarrhée virale bovine (BVDV). La maladie en phase terminale a affecté 21 bouvillons dans la période de 18 à 66 jours suivant la vaccination et a été confirmée comme la maladie des muqueuses après l'examen post-mortem de trois carcasses. Il y avait aussi 17 autres animaux immunotolérants qui demeurèrent sans symptômes durant l'étude. Une étude en laboratoire a été faite afin de déterminer si la maladie des muqueuses a été provoquée par l'un des virus BVDV du vaccin multivalent. Des isolats de virus BVDV cytopathiques purifiés obtenus à partir de deux animaux qui moururent tôt dans la flambée étaient génétiquement identiques (>99%) à la souche 125a du BVDV du type 2 dans le vaccin dans trois régions génomiques : la région 5' non traduite de N^{pro}, E2 et NS2/3. Cette similitude génétique combinée à la présence d'une flambée à un seul pic qui prit place peu de temps après la vaccination suggère que le vaccin BVDV était la cause de la maladie des muqueuses chez ces bouvillons. Cette recherche met en lumière les difficultés de déterminer au laboratoire le rôle du BVDV vaccinal lorsque la maladie afflige plusieurs animaux moins de deux mois suivant la vaccination et le besoin de tests en laboratoire pour identifier les animaux immunotolérants.

Introduction and Background

Bovine viral diarrhea virus (BVDV) is a ubiquitous RNA virus of cattle and other ruminants. There are 2 genotypes, BVDV1 and BVDV2, and multiple subgeno-

types based on the comparison of the 5' untranslated region (UTR), and genomic regions for the N[pro] and E2 proteins.[7,46,53] Within each genotype there are 2 biotypes, cytopathic (cp) and noncytopathic (ncp), based on the characteristic of virus growth in cell culture.[8,43] BVDV-related illness is of great economic importance to producers due to respiratory disease, diarrhea, reproductive losses, and altered immune responses.[11,30] Reproductive losses are due to the ability of the virus to cross the placenta and infect the fetus.[4,23,32] This can result in abortion, malformations, congenital infections, or the live birth of a persistently infected (PI), immunotolerant calf.[8] Persistent infections can only occur if the fetus is infected with ncp BVDV between 1 to 4 months of gestation. PI animals are often unthrifty, and many die before weaning. However, some PI calves are apparently healthy and go unrecognized without laboratory testing. They continuously shed large amounts of the virus and are the main source of disease transmission.[27] In feedlot cattle, direct contact with PI animals has been shown to result in decreased performance and increased fatalities.[30] This makes it critical to identify and cull PI cattle, as well as provide protective immunity through vaccination in order to control and eradicate BVDV in a herd.[17,33,35]

Mucosal disease (MD) is an infrequent, often fatal disease in PI cattle that become co-infected with cp BVDV.[20,36] Experimental infections have been found to cause early- or late-onset MD. Early-onset MD results in disease within 2 to 3 weeks of cp BVDV challenge, and death within a few days. In these cases the cp BVDV virus re-isolated is identical to the challenge virus. When late-onset MD occurs, disease onset is months to years after challenge and the re-isolated cp virus has been found to be a recombination between the persisting ncp and the original cp BVDV.[5,10,18,22,36,37] The classical diagnostic criteria for MD is detection of both ncp and cp BVDV, and a distinctive clinicopathological ulcerative syndrome involving primarily the digestive tract.

Following its first description in 1946,[12] it was 4 decades before the pathogenesis of MD was partly described as severe disease due to the failure to mount an effective antibody response to a co-infection with an antigenically related cp biotype.[6,8,9,36,38] Natural and experimentally-induced cases of MD suggest that other immune mechanisms are also involved in the disease outcome.[6,10,57] In many naturally occurring infections, the co-infecting cp virus originates from a spontaneous mutation in the endogenous ncp virus from a PI animal, resulting in the alternate biotype.[5] The co-infecting virus may also originate from infection with an exogenous cp virus, including modified-live virus vaccines.[26,44] Concern about MD increased when the first live cp virus vaccine was released on the North American market in 1964. Early reports document MD occurring 1 to 4 weeks after administration of vaccines containing modified-live cp BVDV, but studies to show genetic relatedness between the vaccine and cp virus isolated from cases were not done.[16,26,39,41,51] Although lacking specificity, the term 'post-vaccinal MD' became established in the veterinary literature and implies that cases of MD which occur shortly after vaccination (2 to 4 weeks) are a result of the vaccine, on the principal of *post hoc, ergo proper hoc* ('after the fact, therefore because of the fact'). However, persistently infected cattle vaccinated with live BVDV vaccines do not always develop MD. In some cases this has been explained by the extent of antigenic relatedness of the cp and ncp BVDV pair. Given the frequency with which cattle are given vaccines containing live BVDV, a more specific term for MD triggered by vaccinal BVDV might be 'vaccine-precipitated MD.' Delayed-onset post-vaccinal MD has also been reported due to endogenous ncp virus recombining with vaccine cp virus, and causing MD 3 months post-vaccination in the form of classic, late-onset MD.[45]

Routine diagnostic assays for BVDV detect virus antigens or nucleic acid by a variety of methods, including reverse transcription PCR (RT-PCR), virus isolation, enzyme-linked immunosorbent assay (ELISA), fluorescent antibody detection, or immunohistochemistry (IHC). The genotype can be determined by RT-PCR based on the conserved 5 prime untranslated region (5'-UTR).[46,48] Subgenotype determination requires sequencing of the 5'-UTR and/or the adjacent region coding for the protein N[pro].[2,21,48,55] The highly variable E2 gene codes for the major surface glycoprotein.[13] This protein is the main antigenic determinant for inducing neutralizing antibodies.[29,52] The NS2/3 genetic region, also called p125, codes for the protein p54/p80 and is a region of variability, with examples of gene insertions or duplications reported.[1,50,54,56] This region is the genetic basis for many, but not all, changes resulting in the cp biotype.[28,42] The purpose of this investigation was to determine whether a MD outbreak was directly triggered by recent vaccination. To that end, our investigation focused on sequencing to determine the genetic relatedness of modified-live vaccine virus and cp and ncp virus isolated from MD cases from this outbreak.

Materials and Methods

Cattle

Four-hundred and sixty-one short yearling Angus-cross steers were purchased via a video auction and transported directly from the ranch of origin to a backgrounder in southeastern Wyoming. All originated from a single beef herd, where they had received at weaning a multivalent vaccine[a] with killed agents: infectious bovine rhinotracheitis virus (IBRV), noncytopathic BVDV types 1 and 2, and cytopathic type 1, parainfluenza 3

virus (PI3V), bovine respiratory syncytial virus (BRSV), and *Histophilus somni*. On arrival, 2 underweight, sickly animals were sent back to the source herd. The remaining 459 were divided between 2 adjacent pastures with shared water and fenceline between the groups. One day after arrival, all steers were vaccinated with a multivalent respiratory vaccine[b] containing modified-live IBRV, cytopathic BVDV types 1 and 2, PI3V, and BRSV antigens, and 2 avirulent live bacterial agents, *Mannheimia haemolytica* and *Pasteurella multocida*. The strains of BVDV in the vaccine were 1a (Singer) and 2a (125a, derived from the parent strain 125c). A clostridial bacterin-toxoid was given the following day: *Clostridium chauvoei, septicum, novyi, sordelli, perfringens* Types C&D, and *Histophilus somni*.[c] Three weeks later, all steers except for those sorted into a sick pen (n=18, Figure 1, and Table 1, group 1) were revaccinated with a multivalent vaccine[d] containing modified-live IBRV, BVDV1 (cp Singer strain) and BVDV2 (cp 296 strain), PI3V, BRSV vaccine, and *Histophilus somni* bacterin.

Identification of Persistently Infected Steers

All but 5 steers in the purchased group and 59 commingled steers (Table 1, group 2B) were tested for BVDV antigen 19 to 35 days after vaccination using ear notches and a commercial enzyme-linked immunosorbent assay (ELISA) kit[e] following manufacturer's directions, or by examination of notches by immunohistochemistry.[15] Of the remaining 5 steers, 2 were tested using serum and the ELISA kit at 19 days post-vaccination, and 3 steers died prior to testing. In non-PI cattle it is unlikely to detect modified-live BVDV vaccine virus from skin samples after vaccination.[14] Confirmation of PI versus acute infection was made by virus detection at time of necropsy 1 to 5 weeks after initial testing, or by succumbing to death with signs attributed to MD.

Necropsy

Eighteen steers testing positive for BVDV were examined by necropsy and microscopically (Table 3). Three of these were examined after spontaneous death, and 15 were euthanized by barbiturate[f] overdose immediately prior to necropsy. Tissues, serum, and whole blood were collected and tested for the presence of BVDV via ELISA, virus isolation, and RT-PCR testing.

Virus Isolation

The vaccine type 2 parent strain, BVDV2 125c, was purchased from the National Veterinary Service Laboratories[g] and grown on bovine embryonic testicle
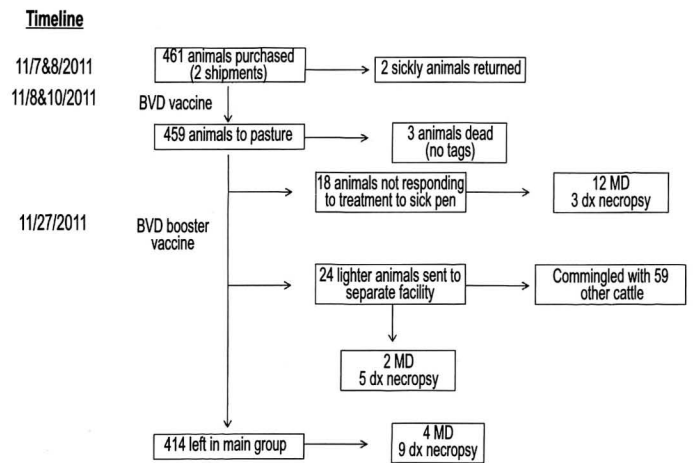

Figure 1. Flow chart depicting the timeline of animal movements, total numbers of animals in the groups, and persistently infected animals that either died from mucosal disease (MD) or were euthanized prior to diagnostic necropsy (dx necropsy).

Table 1. Summary of herd groups showing test status, number of mortalities, days post-vaccination when steers died of mucosal disease, and number necropsied.

Group	N	BVDV status		Natural mortality by day post-vaccination			Necropsy
		Test positive	Mortality*	14-30	30-60	>60	
Early	3	1	2	3			
1	18	15 (83%)		10	2		3
2 A	24	7 (29%)			1	1	5
2 B	59	0 (0%)					
3	414	12 (3%)	1		3	1	9
Total		35	3	13	6	2	17

Early=early mortalities; 1=sick pen; 2A=lightweight steers from source herd commingled with group of 59 other similar-size steers; 2B=separate source steers penned with lightweight steers; 3=main group of steers kept in 2 adjacent fields; *=untested mortalities with clinical signs consistent with mucosal disease

(BeT) cells. Homogenized tissues diluted 1:10 in Bovarnick's media (sucrose, KH2PO4, K2HPO4, glutamic acid, phenol red), or blood and serum diluted 1:10 in Earle's media (199E),[h] were applied to semi-confluent BeT cells in 24 well plates. Plates were incubated at 98.6°F (37°C) for 1 hour in 5% CO^2, washed 1X and maintained in complete media 199E with 2% Pharma-grade FBS.[i] BeT cells and FBS had been previously found negative for BVDV by VI and RT-PCR. Cultures were observed daily for cytopathic effect. Cells were stained for BVDV on days 2 and 9 post-inoculation with monoclonal antibody 20.10.6,[j] followed by fluorescent-labeled goat anti-mouse antibody.[k] Cytopathic virus isolated from the first laboratory-diagnosed case of MD was purified by 4X plaque purification, and partially purified from the second diagnosed case (Table 3, calf numbers 3 and 15).

RNA Extraction

Total RNA was extracted from tissue, blood, cell culture isolates, or monovalent BVDV2 125a binary ethylenimine inactivated vaccine provided by the vaccine company using Trizol Reagent.[l] The isolated RNA was eluted in RNase free dH2O and frozen at -112°F (-80°C) until used in RT-PCR assays. RNA for the vaccine type 1 (Singer c) and type 2 (125a) BVDV was also directly supplied from the company.

PCR and Sequencing

To distinguish BVDV1 and BVDV2, type-specific PCR was run as previously described for the 5'-UTR.[48] PCR products for sequencing were generated from 3 regions of the BVDV genome: 5'-UTR-N[pro] (700 nucleotides), E2 (625 nucleotides), and NS2/3 (502 nucleotides). Primers are listed in Table 2, and regions of the genome sequenced are illustrated in Figure 2. The primers used to generate the 5'UTR-N[pro] region were 01F and N[pro] R, except for cp BVDV isolates and the vaccine BVDV2 125a, for which the alternate 324 forward primer was used (Table 2). Sanger sequencing was performed by Clemson University Genomic Institute,[m] sequencing both strands using the PCR forward and reverse primers. Sequence alignment was performed using commercial sequence analysis software.[n] Nine steers (Table 3) were selected to include a variety of presentations (early MD cases and long-term survivors) for virus identification by sequencing of the 5'-UTR-N[pro] region using RNA extracted directly from blood, tissues, or non-purified cell culture growth. Some of these were also sequenced in the E2 regions. Purified or partially purified cytopathic virus from the first and second laboratory-confirmed cases were sequenced in all 3 regions (Table 3, calf numbers 3 and 15). Sequences obtained were compared with published sequences in GenBank using an online search tool.[o]

Table 2. Primers used for genotyping and sequencing bovine viral diarrhea viruses associated with mucosal disease in steers.

Primer	Sequence 5'-3'	Location, reference sequence
01 F	AGGCTAGCCATGCCCTTAGT	99-118, NADL M31182
324 F	ATGCCCTTAGTAGGACTAGCA	108-128 NADL M31182
N[pro] R	CCATCTATACACACATAAATGTGGT	771-795, NADL M31182
E2 F	GGCCGTGCACGAAAGAGC	195-212, BVDV2 125c E2 AF083345.1
E2 R	GGGTCCCAGGTCAGTGTTAGTAGC	796-819, 125c BVDV2 E2 AF083345.1
NS2/3F	CTACTTTGCGCTGATGGATGGA	396-417, 125c p125, U25053.1
NS2/3R	CGTTTCTAGCCCCCTTCTCACTT	875-897, 125c p125, U25053.1

Primers used for PCR and sequencing are listed. The primer locations are based on published GenBank sequences for BVDV1 NADL, or BVDV2 125c as indicated.
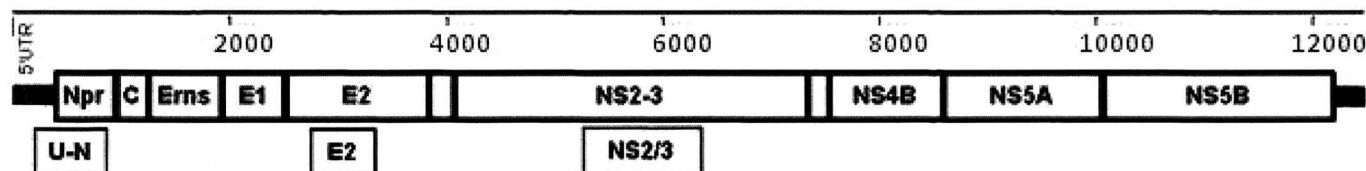

Figure 2. Schematic representation of the approximate 12.4 kb RNA genome of BVDV labeled with the major genes and the terminal 5'- and 3'- untranslated regions (UTR). Sequenced regions in this study (U-N=5'-UTR-N[pro], E2, and NS2/3) are represented as boxed areas underneath the genome.

Table 3. Persistently infected cattle by day of mortality and BVDV sequencing results.

Calf no.	DPV*	Died/ euthanized	Postmortem	MD	Sequencing results 5-UTR-N^pro	Sequencing results E2	Sequencing results NS2/3
1†	16	Died	No	PR			
2†	18	Died	No	PR			
3	19	Died	Yes	+	NCP, 125c§	125c§	125c§
4	23	Died	No	PR			
5	23	Died	No	PR			
6	24-29	Died	No	PR			
7	25	Died	No	PR			
8	26	Died	No	PR			
9	27	Died	No	PR			
10	28	Died	No	PR	NCP		
11	28	Died	No	PR			
12	29	Died	No	PR			
13	29	Died	No	PR			
14†	32	Died	No	PR			
15	38	Died	Yes	+	NCP, 125c§	NCP, 125c§	125c§
16	39	Died	No	PR			
17	47-49	Died	No	PR			
18	48	Died	No	PR			
19	48	Died	No	PR			
20	49	Euthanized	Yes	+	NCP	NCP	
21	49	Euthanized	Yes	No			
22	49	Euthanized	Yes	No	NCP	NCP	
23	66	Died	No	PR			
24	66	Euthanized	Yes	No			
25	66	Euthanized	Yes	No	NCP	NCP	
26	66	Euthanized	Yes	No	NCP		
27	66	Euthanized	Yes	No			
28	72	Euthanized	No	No			
29	72	Euthanized	No	No			
30	73	Euthanized	Yes	No			
31	73	Euthanized	Yes	No			
32	73	Euthanized	Yes	No			
33	99	Died	Yes	UN	NCP	NCP	
34	112	Euthanized	Yes	No			
35	112	Euthanized	Yes	No	NCP	NCP	
36	114	Euthanized	Yes	No			
37	114	Euthanized	Yes	No			
38	114	Euthanized	Yes	No			

35 steers tested positive for BVDV by RT-PCR, VI, ELISA, or IHC, and 3 untested mortalities died with symptoms attributed to MD. All steers died of natural mortality or were euthanized and necropsied as part of the study.
*=days post-vaccination, †=untested for BVDV, §=sequence result of purified or semi-purified cytopathic virus, MD=confirmed mucosal disease, PR=presumed mucosal disease mortality, +=macroscopic lesions consistent with MD, UN=undetermined, NCP=sequence of noncytopathic virus related (97%) to BVDV2 strain SH-28 (GenBank HQ258810.1), 125c=sequence with >99% identity with BVDV2 125c for regions U5'UTR-N^pro, E2; GenBank AF038845.1, and NS2/3 (p125): GenBank U25053.1).

Results

Outbreak Description

Within 10 days of purchase, some animals in the purchased group began exhibiting nasal discharge and variably bloody diarrhea. This led to the entire purchase group being size/condition and health sorted.

Cattle noticeably sick with bloody diarrhea (n=15), lame (n=2), and suspect urolithiasis (n=1) were moved to an isolation pen (Table 1, group 1) and individually treated for coccidiosis with an oral drench.[p] Twenty-four lightweight steers (Table 1, group 2A) were moved to a separate facility where they were commingled with 59 other cattle (Table 1, group 2B) of similar size and age,

sharing pens, fencelines, and water. A flow chart illustrating the disposition of groups is shown in Figure 1.

Two steers died between 2 and 3 weeks after purchase with clinical signs consistent with MD, acute or chronic BVDV, including depression, nasal and oral discharge, mucosal erosions in the oral cavity, and variable bloody diarrhea.[31,58] These were the first deaths in the consignment, and no laboratory samples were collected (Table 3, calf numbers 1 and 2). A third mortality was examined postmortem and had gross lesions typical of MD; it was positive for BVDV by VI and BVDV2 by type-specific RT-PCR (Table 3, calf number 3).

All remaining steers from the consignment, except for 1 additional mortality, were then tested for BVDV, and 34 (Table 3) were test-positive: 15 from the sick pen (15/18, 83%, Table 1, group 1), 7 from the group of sorted lightweight steers (7/24, 29% Table 1, group 2A), and 12 in the main herd (12/414, 3%, Table 1, group 3). None of the 59 commingled steers (Table 1, group 2B) tested BVDV-positive by ELISA.

Over the next 2 months 18 of the remaining BVDV-positive steers died on the farm with signs typical of MD, 10 of these within the 8 days following the initial diagnosis (Table 3). An epidemic curve illustrating the timing of the natural mortalities is shown in Figure 3. Most farm mortalities were not examined (n=16), 1 had confirmed MD based on typical postmortem examination findings (Table 3, calf number 15) and positive VI and RT-PCR, and 1 was undetermined due to predator scavenging, autolysis, and confounding grain overload (n=1, Table 3, calf number 33). The remaining 16 positive steers were free of signs of illness and were euthanized over the next 3 months and examined postmortem. Only 1 of these steers had mild gross lesions attributed to MD (Table 3, calf number 20). Of the 21 presumed or confirmed MD mortalities, 16 had received only the first BVDV-containing vaccine at the backgrounding facility.

Necropsy

Of the steers necropsied, 3 had macroscopic lesions consistent with MD (Table 3, calf numbers 3, 15, and 20). Steers numbered 3 and 15 died on days 19 and 38 after vaccination, while calf number 20 was euthanized on day 49 after the initial vaccination. The main findings were ulcers in the tongue, lips, oral cavity, esophagus, rumen, Peyer's patches, cecum, and colon (Figure 4). One animal had a secondary fungal infection of the intestinal ulcers, and another had marked enteritis with crypt necrosis. The confirmatory diagnosis of MD was based upon typical macroscopic and microscopic lesions, the identification of BVDV by RT-PCR, and isolation of cp and ncp virus. A fourth animal (calf number 33) in poor postmortem condition (predator scavenged and autolytic tissues) had lesions suggestive of MD or ruminal acidosis/grain overload. The remaining steers had minimal

gross and histological lesions; no other bacterial or viral pathogens were found as significant factors.

PCR and Sequencing

Blood and tissue samples from all of the necropsied steers (n=18) were positive for BVDV2 by type-specific RT-PCR (data not shown). Sequences of ncp virus isolates and RNA directly from blood or tissues of 9 steers (Table 3) were identical to each other and most closely related (97% identity) to BVDV2 strain SH-28 (GenBank HQ258810.1) in the 5'-UTR – N[pro] and E2 regions (sequences submitted to GenBank; accession numbers KC596021, KC596022). We sequenced the 5'UTR-N[pro]
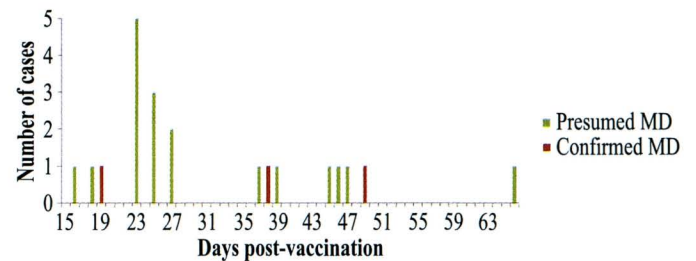

Figure 3. Epidemic curve for the outbreak of mucosal disease (MD) in a Wyoming cattle herd. The natural mortalities presumed or confirmed by laboratory testing as MD are illustrated.

Figure 4. Images from the index case (Table 3, calf number 3) of mucosal disease following vaccination 19 days earlier; cytopathic BVDV2 125c BVDV was isolated from tissues. A. Multiple shallow ulcers on dorsum of tongue. B. Ulceration of gut-associated lymphoid tissue with adherent diphtheretic membrane. Histologically there was superimposed mycotic infection throughout this ulcerated lymphoid tissue. C. Focal ulceration of larynx overlying vocal ligament. D. Gingivitis with recession of gum line, exposing roots of cheek teeth.

region of the reference BVDV2 125c strain (submitted to GenBank; accession KC596020) to compare to the vaccine virus BVDV2 125a and our cp isolates. For cp BVDV isolates and vaccine BVDV2 125a, we used the alternate forward primer (Table 2, primer 324) for the 5'UTR-N^{pro} product. This alternate primer overlaps the 01F primer, but is shifted 3' by 10 nt. Based on our sequencing results, there is a single base deletion at the 3' end of the 324 primer, and it appears there are additional changes under the 01F primer that prevent efficient amplification using this primer. Non-purified cp virus from 1 of these steers (Table 3, calf number 15) sequenced as BVDV1 Singer when the 01 forward primer was used instead of the 324 primer.

The genomic regions of E2 and NS2/3 were sequenced to further verify the identity of the cp virus isolates from the first 2 cases (Table 3, calf numbers 3 and 15). These isolates were identical (>99%) to each other, to the vaccine BVDV2-125a, and to published BVDV1 125c sequences in GenBank (Accession numbers: AF083345.1 and U25053.1).

Virus Isolation

Only ncp virus was isolated from blood or serum samples, except for 1 steer from which both biotypes were obtained (data not shown). Viruses isolated from tissue samples collected at necropsy included both cp and ncp cell culture characteristics, and both biotypes could be identified in all animals necropsied (n=18) as late as 3 months after the outbreak without evidence of disease (data not shown). This agrees with previous reports finding long-term presence and shedding of cp virus in PI animals, even when co-infected with a heterologous virus.[22] Such a co-infecting virus has been shown to be the source for genetic recombination with endogenous virus, resulting in late-onset MD.[45]

Discussion

Cattle persistently infected with BVDV that survive past weaning are at risk of dying from MD when co-infected with a cp virus. There are 3 main sources of cp BVDV causing MD: 1) spontaneous mutation of ncp virus resulting in the cp biotype, accounting for most episodes of MD in herds and includes recombination with field or vaccine virus, 2) exposure to an exogenous field strain cp BVDV, or 3) exposure to exogenous cp virus in a live vaccine.[3,5,40] Determining the source of cp virus causing MD goes beyond routine diagnostic testing, since it requires purification steps to separate the ncp and cp BVDV from cell cultures, followed by sequencing to identify the specific virus.[3,24,25,34]

The vaccine BVDV2 125a and cp isolates were difficult to amplify by RT-PCR in the 5'-UTR region, and required a different forward primer (Table 2, primer 324)

due to a polymorphism at the forward priming site. This was the likely cause of finding type 1 BVDV Singer strain in a non-purified cp isolate from 1 steer (Table 3, calf number 15) when this region was sequenced using the alternate (01) forward primer, while the E2 and NS2/3 regions gave sequences identical to reference BVDV2 125c and the vaccine. This calf had received 2 doses of vaccine containing type 1 BVDV Singer, vaccinated on days 1 and 21.

Our sequencing results demonstrate genetic identity (>99%) between the cp virus isolated from the first 2 diagnosed MD cases (Table 3, calf numbers 3 and 15) and the vaccine BVDV2 virus, indicating MD in these steers was probably directly triggered by the vaccine. There was not close genetic identify between the ncp and cp BVDV in the genomic regions that were sequenced, and antigenic relatedness was not tested in this study. Antigenically homologous virus pairs with failure to mount an antibody response have been found in many cases of MD;[8] however, antigenically and genetically unrelated viruses have been found in experimental and naturally occurring cases of MD.[5,10,47,49,57] It is likely that a combination of viral and host factors determine the clinical outcome.

The temporal pattern of cases illustrated in the epidemic curve (Figure 3) was consistent with a point-source exposure, with cases beginning 2 weeks after vaccination. Although many early mortalities were not examined by necropsy, the timing of the mortalities in PI steers with identical clinical signs supports a presumptive diagnosis of MD. The short incubation from vaccination to mortalities is unlike the pattern that is seen in delayed-onset post-vaccine MD after genetic recombination occurs between a live vaccine and the endogenous ncp virus.[3,45] It is not possible to completely exclude the possibility of endogenous ncp virus mutation or recent exposure to field strains of cp BVDV2 125c. In either of these situations, the temporal pattern of cases should have exhibited delayed-onset or multiple peaks rather than a single cluster (within 2 months) after vaccination.

From this group of 459 steers, 35 tested positive for BVDV (1 by necropsy and 34 by ELISA or IHC), and 2 early and 1 late untested mortalities were presumed positive. All positive steers not dying of presumed MD on the farm were necropsied 1 to 5 weeks after initial testing and found to be BVDV positive by both PCR and VI, thereby confirming PI status. It is noteworthy that almost half (n=17) of the PI animals that had the same ncp virus based on the 5'-UTR-N^{pro} and E2 regions did not develop MD as late as 3 months post-vaccination. Late onset, chronic, or transient MD has been reported, but the cause of the variable outcome from classic acute MD is not clear.[8,10,18]

This outbreak illustrates the potential risk that exists when PI cattle are vaccinated with cp modified-

live virus vaccine. The occurrence of presumptive post-vaccinal MD should trigger a search for additional persistently infected asymptomatic animals so that they can be eliminated from the cohort. Sorting of sickly and small animals was partially successful in separating PI animals from the rest of the herd, removing 25 of the 38 (66%) PI animals and reducing the group prevalence from 8% to 3%. However, sorting based on physical characteristics still missed 13 animals that would have contributed to BVD persistence within the herd had they not been detected by laboratory testing. The precipitation of MD by vaccination led to laboratory testing, identification, and culling of the other PI cattle, and likely prevented further BVD losses in the herd.

The benefit of vaccination against bovine respiratory complex agents, including BVDV, is well known.[35] The use of modified-live virus vaccine may be preferred over killed virus products when there is need to produce a faster and broader immune response. Despite direct contact with PI cattle shedding large amounts of virus, there was no noticeable evidence of acute BVD illness in non-PI steers. This may have been due to protection elicited by the vaccine,[19] though a controlled study with non-vaccinated naïve controls would be needed to prove this point.

Conclusions

Collectively, the molecular and epidemiologic findings are consistent with a vaccine origin in this MD outbreak. Recognition of MD 2 to 4 weeks after vaccination with modified-live cp virus should prompt testing of the entire group to identify persistently infected animals so that they can be isolated or culled. It is more important to identify PI cattle than to establish whether vaccine triggered MD, since these PI animals shed a large amount of virus, and some of them may proceed to develop terminal disease due to mutations of the endogenous ncp BVDV. This case demonstrates the utility of a 3-pronged approach to BVDV biosecurity: 1) laboratory testing to identify PI animals, 2) separation or culling of positives, and 3) vaccination to protect naïve animals.

Endnotes

[a]Virashield 6 + H. Somni, Novartis Animal Health, Basel, Switzerland
[b]Vista Once, Merck Animal Health, Whitehouse, ME
[c]Vision® 7 Somnus, Intervet Inc., Millsboro, DE
[d]Express 5 HS, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
[e]Idexx Herdchek BVDV test kit, Idexx Laboratories, Inc., Westbrook, ME
[f]Beuthanasia-D Special. Merck Animal Health, Summit, NJ
[g]National Veterinary Service Laboratories, Ames IA
[h]Earle's media (199E), Sigma, St. Louis, MO
[i]Pharma-grade FBS, PAA Laboratories Inc, Pasching Austria
[j]Monoclonal antibody 20.10.6, Dr. Ed Dubovi, Cornell University, Ithaca, NY
[k]Fluorescein labeled goat anti-mouse antibody, Invitrogen, Carlsbad, CA
[l]Trizol Reagent, Gibco BRL, Gaithersburg, MD
[m]Clemson University Genomic Institute, Clemson, SC
[n]SeqMan Pro, DNASTAR, Lasargene software, Madison, WI
[o]Basic Local Alignment Search Tool (BLAST). National Center for Biotechnology Information, National Library of Medicine. Online at http://blast.ncbi.nlm.nih.gov/Blast.cgi
[p]Corid, Merial Limited, Dublin, GA

Acknowledgements

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