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SEROLOGICAL EVALUATION OF SENTINEL CALVES IN A BVDV ERADICATION PROGRAM

By

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ABSTRACT

SEROLOGICAL EVALUATION OF SENTINEL CALVES IN A BVDV ERADICATION PROGRAM

By

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The identification and removal of persistently infected (PI) cattle is critical to eradicating bovine viral diarrhea virus (BVDV). Serological evaluation of small groups of young unvaccinated calves has been proposed as an alternative method for identifying herds with cattle persistently infected (PI) with BVDV. The objective of the study reported here was to evaluate the application of sentinel serology as an on-going herd monitoring tool in herds enrolled in a regional BVDV eradication project.

Serum samples were collected from 47 management groups from 36 herds. Virus neutralization (VN) antibody titers to type 1 and type 2 BVDV were determined from five non-vaccinated calves > 6 months of age in each management group. A management group was considered to be BVDV positive if 2 of 5 calves had VN titers greater than $\geq$ 128. Skin samples from all calves in all herds were analyzed using reverse transcriptase polymerase chain reaction (RT-PCR) to identify PI animals from any infected herds.

PI cattle were identified in one herd with one management group. In that herd, 3 sentinel calves had VN antibody titers $\geq$128. All other management groups were negative for BVDV. The κ value for agreement between sentinel serology and RT-PCR was 1.0 (95% CI 1.0 to 1.0). This study further supports the use of sentinel serology for identifying cattle herds with BVDV and its potential use as a tool in BVDV eradication programs.

DEDICATION

It is my honor to dedicate this work to my parents
Keith and Adrienne Corbett.
Thank you for all of your love and support throughout the years,
especially during the time when I was searching for the
right career in veterinary medicine.

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I would like to thanks my graduate committee members, Drs. Dan Grooms, Steve Bolin, and Ben Bartlett, for their guidance and support in completing this project. I would especially like to the Dr. Grooms for providing me with the opportunity to return to graduate school and for putting up with me the past year and a half. You have been a great mentor and friend to me and I will attempt to carry all that I've learned into my future endeavors.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 3

KEY TO ABREVIATIONS

ACE	Antigen Capture ELISA
BRD	Bovine Respiratory Disease
BVDV	Bovine Viral Diarrhea Virus
BVD	Bovine Viral Diarrhea
CI	Confidence Interval
CPE	Cytopathic Effects
DCPAH	Diagnostic Center for Population and Animal Health
DMEM	Dullbecco's Modified Eagle Medium
EED	Early Embryonic Death
ELISA	Enzyme Linked Immunosorbent Assay
IHC	Immunohistochemistry
MLV	Modified Live Virus
RCF	Relative Centrifugal Force
PI	Persistently Infected or Persistent Infection
RT-PCR	Reverse Transcriptase - Polymerase Chain Reaction
UTR	Untranslated Region
VI	Virus Isolation
VN	Virus Neutralization

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is one of the most economically devastating diseases of cattle, affecting animals throughout the world. Control of bovine viral diarrhea virus (BVDV) has been a worldwide challenge for decades. Various clinical manifestations can result from infection with BVDV, ranging from subclinical infection to immunosuppresssion and respiratory disease to abortion and infertility. The most well known source for transmission of BVDV are persistently infected (PI) cattle. Persistent infection occurs when calves are exposed to BVDV *in utero* before the fetal immune system is developed, thus allowing the developing fetus to become immunotolerant to the virus. PI cattle continuously shed large amounts of virus into the environment and are an important source of virus transmission within and between herds.

In 2007, the Michigan Upper Peninsula BVDV Eradication Program was launched. The purpose of this project is to eradicate BVDV from a geographic area and, in doing so, identify benefits and obstacles of a BVDV eradication program and demonstrate a feasible model that may be adopted by other parts of the US. Key components of the program include identification and removal of cattle persistently infected with BVDV, institution of a planned biosecurity program, and appropriate BVDV vaccination. Whole herd testing to identify cattle persistently infected with BVDV is a major investment of both time and money for producers. Therefore, development of more efficient herd testing strategies would be beneficial. Serological evaluation of small groups of young unvaccinated calves has been proposed as an alternative method for identifying herds with cattle persistently infected (PI) with BVDV.

The objective of the study reported here was to evaluate the application of sentinel serology as an on-going herd monitoring tool in herds enrolled in a regional BVDV eradication project. The goal of sentinel testing is to provide an effective herd screening tool that can be used in follow-up surveillance in an eradication program.

CHAPTER 1
LITERATURE REVIEW

Introduction

Bovine viral diarrhea virus (BVDV) is an important pathogen affecting cattle worldwide and is one of the most economically devastating diseases of the cattle industry. The initial clinical disease, named Bovine Viral Diarrhea (BVD), was first described in the United States in 1946 and was associated with gastroenteritis and severe diarrhea, with erosive lesions of the digestive tract.[1] The disease was associated with high morbidity and low mortality rates. Since then the virus has been isolated throughout the world from a variety of animals including cattle, sheep, llamas, and deer. Infection with BVDV can manifest itself in several different syndromes including diarrhea, respiratory disease, immunosuppression, subclinical infection, and reproductive failure. Causes of reproductive failure associated with BVDV include infertility, early embryonic death (EED), abortion, and congenital defects. The virus can also impair the immunity of infected animals which can aggravate other diseases or increase the susceptibility to other disease complexes such as bronchopneumonia, diarrhea, and mastitis. One of the more unique characteristics of BVDV is its ability to create persistently infected (PI) cattle as a consequence of *in utero* infection by noncytopathic BVDV between days 40 and 125 of gestation. Infected fetuses that survive until birth are born immunotolerant to the specific exposing viral strain and are lifelong shedders of the virus. PI cattle are the principle reservoir of BVDV and serve as the primary source of viral spread.

The following review will review in further detail what is known about BVDV: the types of infection, prevalence and incidence of the disease, virus transmission, diagnostic strategies, control and prevention, and eradication.

The Virus

Bovine viral diarrhea virus is a positive-sense, single-stranded RNA virus. It belongs to the genus *Pestivirus* in the *Flaviviridae* family. BVDV isolates are further broken down by their genotype and biotype.

In 1993, there were sudden deaths among adult dairy cattle in Canada and parts of the northeastern U.S.[2] Genetic typing found that these BVDV isolates were quite different than the recognized strains in existence.[3] With the advent of polymerase chain reaction (PCR) in the 1990's, it became possible to differentiate BVDV into two different genotypes: type 1 and type 2. These genotypes were further broken down into subgenotypes. Currently there are 11 subgenotypes of BVDV type 1 (a-k) and two subgenotypes of BVDV type 2 (a and b).[4] The most common subtypes in North America are type 1a, 1b, and 2a.[5] Type 1 strains are most commonly associated with subclinical or mild respiratory disease.[4] Type 2 isolates tend to be associated with more severe disease, including hemorrhagic syndrome. BVDV type 2 represents up to 50% of laboratory isolates in North America.[6-9] A recent study of BVDV isolates in North America and Australia summarized data from around the world suggesting that BVDV type 2 is rare in countries outside of North America.[10]

Within each genotype of BVDV exist two biotypes. The biotype of the virus refers to the ability of the virus to cause cell pathology in infected cell cultures. Cytopathic virus causes cell death in vitro, while noncytopathic virus causes no visible cell damage. Studies have shown that most BVDV field isolates are noncytopathic.[7,11] Noncytopathic stains of BVDV also tend to be more virulent.[11]

Cytopathic BVDV was discovered several years after noncytopathic isolates and it is believed that cytopathic BVDV evolved as a mutant strain of a noncytopathic virus.[12] To date, only noncytopathic BVDV has been isolated from PI cattle.[13,14] Since PI cattle typically represent the primary reservoir of BVDV within the population, it would suggest that noncytopathic BVDV is the natural form of the virus, while cytopathic BVDV strains are an atypical form.

Acute Infection with BVDV

Acute BVDV infection refers to infection that occurs in cattle that are not persistently infected. Most BVDV infections are subclinical in nature and therefore go undetected.[15] These cattle may develop a mild fever and become leukopenic prior to developing serum-neutralizing antibodies and clearing the virus.

Clinical infection tends to occur in young cattle between six months and two years of age. After a 5-7 day incubation period, affected cattle develop high fevers and severe leucopenia. Clinical signs may include fever (40-41 °C), oculonasal discharge, depression, anorexia, decreased milk production, transient watery diarrhea, and erosions or shallow ulcerations in the oral cavity. Additional signs can occur sporadically. The

course varies from 2-3 days up to 3 weeks with high morbidity and low mortality. Affected animals shed the virus for up to 15 days post infection.

Bovine viral diarrhea virus is an important component of the bovine respiratory disease complex (BRD). The primary role of BVDV in BRD is likely immunosuppression. The leukopenia that arises from infection with the virus destroys or inhibits the normal function of white blood cells and allows for secondary bacterial pathogens an ideal environment to establish infection.

As mentioned previously, type 2 BVDV was first isolated from disease outbreaks in Canada and in the northeastern U.S. in 1993.[2,16] Infections with type 2 BVDV during these outbreaks resulted in mortality rates as high as 20-25% in individual herds. Clinical signs include marked thrombocytopenia, bloody diarrhea, epistaxis, hyphema, bleeding from injection sites, pyrexia, pneumonia, and severe hemorrhage throughout the body. This disease manifestation is referred to as hemorrhagic syndrome.

Fetal Infection with BVDV

Fetal infection occurs when a pregnant dam becomes acutely infected with BVDV or when a persistently infected dam becomes pregnant. The result of BVDV fetal infection depends on the stage of gestation in which the fetus is infected, as well as the virulence of the virus.

BVDV infection prior to 50 days of gestation

Infection at the time of insemination or shortly after has been associated with low conception rates.[17] The reason for low conception rates is not clear but may depend on

the stage of early reproductive events and the time of infection. One reason for lower conception rates during the first weeks of gestation is EED and resorption of the embryo. The cow will become a repeat breeder and will return to estrus after a prolonged period of time.

Another reason for lower conception rates could be that a transient endometritis is created by the virus, resulting in inhibition of normal embryonic implantation.[18] In addition, virus has been isolated in ovarian tissue following acute infection with both cytopathic and noncytopathic BVDV.[19-22]

Infections in the first 50 days are often unnoticed without careful monitoring at the herd level. Increase in the number of services per conception and irregular estrus cycles are suggestive of EED, and BVDV should be considered as a possible cause.

BVDV infection 51-100 days of gestation

Fetal infection 51-100 days into gestation can result in fetal death followed by abortion or mummification.[17,23] Several weeks or months may pass before the fetus is expelled from the cow.

Abortions caused by BVDV can be difficult to diagnose and many aborted fetuses associated with BVDV are negative on virus isolation.[13] A major contributing factor is the delay in fetal expulsion following BVDV infection. Because of the delay, BVDV is often no longer present in the fetus. In addition, cows have often seroconverted by the time of fetal expulsion , making acute and convalescent titers of little value.[24]

BVDV infection 101-150 days of gestation

Nervous system congenital defects are a concern following transplacental infection between 101-150 days in gestation.[17] During this period the fetus is in the final stages of nervous system development. The inflammatory response to BVDV from the fetal immune system can injure the developing nervous system, leading to inhibition of cellular growth, cell differentiation, or cell lysis.[13] The most common defect associated with BVDV infection is cerebellar hypoplasia. Difficulty standing, a wide based stance, and intention tremors are associated with cerebellar hypoplasia in calves.[25,26] Other congenital defects seen include ocular problems (blindness, retinal degeneration, and cataracts) and skeletal defects (small malformed calves).

BVDV infection after 150 days of gestation

After approximately 150 days of gestation, immunocompetence and organogenesis are usually complete in the developing fetus. Abortion and premature births can occur in cases in which the infecting virus is particularly virulent.[25,27] Transplacental infections with BVDV at this point often result in the birth of normal calves seropositive to BVDV.[17] However, one study suggests that congenital infections during the last trimester of gestation may have a negative effect on neonatal performance and survivability, as calves born with BVDV-neutralizing antibodies were twice as likely to experience severe illness within the first 10 months of life.[28] In addition, a recent case study of an outbreak of late term abortions and premature births associated BVDV

identified brachygnathism, thrombocytopenia, malformations of the brain and cranium, and rare extracranial skeletal malformations.[29]

Persistent infection with BVDV

Persistent infection with BVDV occurs when the fetus is exposed to noncytopathic virus between days 18 and 125 of gestation.[13,17] Fetal infection during this window is the only known way of creating persistently infected cattle. It occurs during the period when the immune system is identifying self-antigens and virus circulating is recognized as a normal part of the fetus. The fetus becomes immunotolerant to the virus and cannot clear the virus.[30] The calf is born a lifelong carrier of the virus and is referred to as being persistently infected or PI.

PI's have virus in every cell of their body and shed large amounts of virus in all excretions and secretions during its life.[30,31] PI calves may appear clinically normal and survive well into adulthood, but most are small and unthrifty.[32-34] They often are culled or die prior to becoming adults. There is no cure for PI's.

PI cattle serve as the major source of BVDV transmission both within and between herds.[14,15,35-39] They are continuously shedding virus throughout their lives, serving as a source of infection for naive herd mates. The few PI cattle that survive to adulthood and give birth produce offspring that are also PI's because the virus is present during 50 and 125 days of gestation.[40]

To date, only noncytopathic strains have been isolated from PI cattle and BVDV immunotolerance is strain specific.[12,17,37] PI calves will not form virus neutralizing

antibodies to the BVDV strain that they are immunotolerant to. However, it does not preclude them from becoming infected with a heterologous BVDV strain and subsequently developing antibodies to that strain.

Mucosal Disease

Mucosal disease occurs in PI animals that become super-infected with a cytopathic BVDV strain that is homologous to its immunotolerant noncytopathic strain.[41] These cattle have both noncytopathic and cytopathic BVDV isolated from them, but they typically have no viral neutralizing antibodies. The most likely origin of the cytopathic strain is within the PI animal as a mutation of the persistent noncytopathic strain. Other potential sources are external and included either a cytopathic field strain or modified-live vaccine strain.[13]

Clinical signs of mucosal disease include fever, depression, anorexia, weight loss, and severe dehydration. Physical examination can reveal oral lesions on the lips, gingival margins, tongue, and dental pad; they are often accompanied by ptyalism. Large areas of mucosal necrosis and sloughing are present if the lesions ulcerate. Ulcerating lesions may also be present on the vulva, teats, and interdigital areas resulting in lameness. Intermittent to chronic diarrhea may be present. The disease is often acute with death in 3-10 days after onset of clinical signs. Postmortem examination of the gastrointestinal tract reveals areas of necrosis and mucosal sloughing throughout (esophagus, rumen, abomasums, duodenum, jejunum, ileum, cecum, and colon).[12,13,42]

Occurrence of mucosal disease is sporadic, with mortality approaching 100% in affected cattle.[41] Outbreaks have also been reported in herds where there is a cohort of PI

calves. In this situation, the virus in one PI calf mutates into a cytopathic virus, which then infects its PI herd mates.[43,44] Vaccine-associated outbreaks of mucosal disease have also been reported, as most commercial modified-live BVDV vaccines contain cytopathic virus.[41]

Prevalence of BVDV Infection

BVDV infection has been found in cattle worldwide. The prevalence of BVDV infections is often reported as the percent of PI cattle or the percent of seropositive cattle in a population.[45] It has been estimated that 4% of cow-calf herds and up to 15% of dairy herds in the United States have persistently infected (PI) animals in them.[39,46-48] Seroprevalence studies looking at BVDV antibodies have been conducted throughout the world; with a wide range in seroprevalence (15-89%). Herd seroprevalence can be affected by use of vaccination and may not reflect true disease prevalence. At the individual animal level, PI prevalence ranges from 0-1.7% (Table 1.1).[32,34,39,46,47,49-53] Prevalence of PI's can be higher in individual farms where BVDV control programs are lacking.

Table 1.1 Prevalence of BVDV PI cattle in various studies

Location	Source of Population	# of PI cattle	Total # of cattle	% of PI cattle	Total # of herds	% of PI herds	Ref.
Nebraska, Kansas	Calves and cows	54	3157	1.7%	66	4%	39
Michigan	Dairy herds	71	5481	1.3%	20	15%	37
Saskatchewan	Feedlot calves	5	5129	0.1%	1	100%	25
Alabama, Nebraska, Nevada, North Dakota, Ohio	Beef herds	56	18,931	0.03%	128	4%	31
Texas	Feedlot steers	6	2000	0.3%	1	100%	27
Missouri	Feedlot calves	3	938	0.32%	2	100%	43
Kansas	Feedlot calves	86	21,743	0.4%	1	100%	41
Oklahoma and Texas	Beef calves	25	45300	0.55%	30	16.7%	42
Iowa	Beef calves	12	12,030	0.009%	102	4%	36
Michigan	Beef and dairy herds	0	1549	0%	49	0%	40

Transmission of BVDV Infection

The most efficient means of spreading BVDV is by direct contact with infected animals, but indirect contact can also occur. Virus has been isolated from nasal swabs, aerosols, saliva, milk, urine, feces, semen, and uterine fluids. Transmission of BVDV can be either vertical or horizontal. Vertical transmission from dam to offspring via prenatal infection is extremely efficient, as discussed above.

The most efficient method of horizontal transmission of BVDV is nose-to-nose contact. Inhalation or ingestion of the virus is the most common mode of infection. Because PI's shed large amounts of virus for their entire life, they are considered the major source of BVDV transmission both within and between herds. Cattle that are acutely infected are also an important source of transmission of virus. Acutely infected cattle shed lower levels of virus compared with PI cattle, and they typically shed the virus for 2-10 days. Other ruminant species can become infected with BVDV and potentially serve as a source of transmission. This can include sheep, camelids (llamas and alpacas) and cervidae (deer and elk).[54-58]

Indirect transmission of BVDV involves an intermediate vector that transmits the virus from infected to susceptible animals. Examples of indirect transmission of BVDV that have been documented include contaminated vaccines or health products, contaminated needles, biting flies, and other fomites including contaminated equipment, feed, and people. However, the virus is easily inactivated outside the host, suggesting that indirect transmission plays a minor role in virus transmission.

Transmission of BVDV has been demonstrated to occur between small ruminants and cattle, as well as between cervidae and cattle. BVDV has been isolated from pigs and

a wide variety of ruminants. Clinical disease has been documented in sheep, alpacas, and white-tailed deer.[54-59] A recent study in Alabama showed that when pregnant deer were in cohabitation with persistently infected cattle, their offspring were born infected with BVDV.[55] This was the first report of BVDV transmission from cattle to white-tailed deer using a model of natural challenge. Buffalo and wildebeest with no known contact with cattle have had high BVDV titers detected, suggesting that a wildlife reservoir for BVDV exists.[60-62] The importance of such a reservoir in regards to transmitting infection to domestic cattle is unknown.

Economic Importance of BVDV Infection

Bovine viral diarrhea has been reported to be one of the most economically important diseases in cattle throughout the world.[13,45] In addition to losses from fetal infection (including PI calves and abortions), acute infection can also lead to economic loss. Losses due to acute forms of BVDV infection include reduced milk production, reduced conception rate, respiratory disorders and death.[45] Through immunosuppression, BVDV can intensify the effects of BRD pathogens including *Mannheimia hemolytica*, bovine herpesvirus-1, and bovine respiratory syncytial virus.[63-67]

In the dairy industry, several studies have looked at the economic costs associated with BVDV infections. Economic losses have been calculated in Canada at $2,421 per 50 head of cattle.[68] Estimates of economic losses from infection with highly virulent strains range from $40,000 to $100,000 per herd.[69,70] Other studies have estimated losses at the population level ranging from $10-40 million per million calvings.[36,71]

To date, only two studies have examined economic effects in the beef industry. The first was a 10 year study that looked at a farm profitability model.[72] This study calculated an average return to fixed cost of $20.16 less for farms with at least 1 PI in the herd due to reproductive and calf mortality effects. A more recent study evaluated the economic effects and health and performance of the general cattle population in a starter feedlot after exposure to PI cattle.[73] This economic analysis of this study revealed that fatalities accounted for losses of $5.26/animal and performance losses were $88.26/animal, suggesting that there is a detrimental impact from exposure to BVDV PI cattle in a feedlot.

Diagnosis of BVDV Infection

Many tests are available to detect BVDV. Test selection is often based on cost and availability of these tests. The type of tests available can be broken down into tests available for detecting individual cattle infected with BVDV and tests available for detecting infection at the herd level. The identification and removal of BVDV PI animals from the herd is a key component for controlling of the disease. Several strategies are available for screening herds for PI's.

Virus Detection Tests

Individual animal tests are available to diagnose BVDV in the herd. Testing methods available include virus isolation (VI), immunohistochemistry (IHC), antigen-capture enzyme-linked immunosorbent assay (ACE), and reverse transcriptase polymerase chain reaction assays (RT-PCR). Choosing a diagnostic test is based on cost,

availability, type of specimen needed, and what type of infection that you are trying to detect (acute or PI).

Culture and identification of BVDV using VI remains the 'gold standard' diagnostic technique. [74,75] Several different VI protocols have been developed using three different cell lines: bovine turbinate, bovine testicle and Madin Darby Bovine Kidney. The best sample for BVDV isolation in the live animal is the buffy coat (white blood cells) from a whole blood sample, while lymphoid organs are the best necropsy specimens. Virus isolation can be affected by colostral immunoglobulins, therefore testing should only be done on calves > 2 months of age.[24]

Detection of BVDV antigen from samples is much quicker and cheaper than VI. Two methods are available, antigen capture ELISA's and immunohistochemistry. IHC was the first test used to detect BVDV antigen in the skin and is still widely used as a screening method for identifying PI cattle.[76] IHC on formalin-fixed paraffin-embedded tissues has been shown to be a more sensitive detection method for BVDV antigens in the skin of cattle when compared with VI.[77-79]

More recently, ACE has become one of the predominant screening tests due to its high sensitivity in detection of PI's and ease of use. Serum or skin samples can be used. Commercially available test kits exist that use monoclonal antibodies to capture viral antigen E (gp48) and detects antigen-antibody complexes with enzyme-conjugated antibody by spectrophotometer.[80] Previous studies evaluating the use of ACE on skin samples have shown an high sensitivity and specificity in detecting persistently infected animals.[81,82] Although ACE has been shown to be useful in screening for PI cattle, the

procedure lacks the sensitivity needed to detect most acute BVDV infections in cattle.[75] In addition, results may be inhibited by passive immunity and is not recommended for young calves.[83]

RT-PCR is a highly sensitive diagnostic test that detects pestivirus RNA.[74] RT-PCR can use skin, serum, or whole blood samples. The high analytical sensitivity of RT-PCR allows for pooling of specimens.[75] Equipment costs to run RT-PCR in a laboratory setting can be high, but the procedures are readily adapted to pooled sampling strategies, which reduce test costs to producers. This is especially true when testing for PI cattle, where studies have detected 1 positive serum sample in pools of 50 and 100 samples and 1 positive animal among 99 negative was detected using pooled supernatants from ear notch samples.[84,85] A latter study also calculated a sensitivity of 100% and specificity of 97.5% when using pooled RT-PCR to detect persistently infected cattle.[86] The sensitivity of the RT-PCR assay also increases the likelihood of detecting acute infections.[87] RT-PCR assay of pooled samples is the diagnostic test of choice of the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH) for BVDV PI screening.

Herd Tests

Tests are available for detecting infection at the herd level without testing the whole herd. This can be accomplished by serological evaluation of sentinel animals, bulk tank RT-PCR, or bulk tank antibody testing.

Serologic evaluation of sentinel animals

Sentinel animals are a representative sample of population or herd that serve to determine the disease status of the herd. Analyzing BVDV neutralizing antibody titers of a small group of young unvaccinated heifers has been shown to accurately identify herds infected with BVDV. A high prevalence of seropositive heifers in a herd is indirect evidence that a PI animal is present in the herd. These animals serve as sentinels for circulating virus, suggesting the presence of a PI herd mate.

Initial studies in Denmark and Michigan used a hyper geometric probability function for herds with and without PI cattle to calculate the probability of obtaining seropositive animals. The Danish study looked at the probability of obtaining at least two out of five seropositive animals 6-18 months of age. The probability was 0.977-1 in 10 herds with PI cattle and 0-0.048 in nine herds without PI cattle.[88] The use of a cutoff of at least two of five calves being seropositive resulted in a sensitivity of detecting persistently infected cattle in the herd of 97.7% and a specificity of 95.2%. The Michigan study determined that the probability of a PI animal being present in a herd was 0.99 if three out of five animals had BVDV antibody titers $\geq$128. The study further calculated the probability of a PI animal being in the herd was < 0.01 if three of the five animals had titers $\leq$64.[89] The use of a cutoff of at least three of five calves being seropositive resulted in a sensitivity of detecting persistently infected cattle in the herd of 99.4% and a specificity of 99%. In both of these studies, all cattle had been tested for both BVDV and BVDV antibodies prior to the study.

To date, two studies have looked at implementing sentinel animals in herds of unknown BVDV status to identify herds that contain cattle persistently infected with

BVDV. A study of dairy herds looked at 14 herds and used 5 unvaccinated 6-12 month old heifers as sentinels.[90] A herd was classified as likely to contain PI cattle when at least three out of five heifers had antibody titers ≥128. The herd sensitivity for the serological test was 66% and the specificity was 100%. A separate study looked at 38 beef herds and collected samples from 30 unvaccinated calves.[91] This study determined the optimum sentinel sample size was 10 animals per herd. A herd was classified as likely to contain PI cattle when at least three out of ten calves at weaning had antibody titers ≥1000. The herd sensitivity for beef herds was 53% and specificity was 80%. The authors concluded that serological evaluation of a small number of calves could not be used to accurately predict the presence of PI cattle in a herd. The authors cited potential misclassifications (vaccination, passive antibodies, comingling with other herds) and environmental factors (lack of comingling between management groups, low stocking density) as possible reasons for the lower sensitivity/specificity in their study.

Sentinel testing provides a representation of the entire herd without the cost and time associated with whole herd testing. It can predict the presence or absence of a current infection. Unvaccinated calves aged 6-12 months are typically used in sentinel testing programs.[89] Cattle in this age group have typically cleared passively derived antibodies that could interfere with testing.

One potential negative to sentinel testing is herd misclassification. A false positive test may occur if the PI animal is removed from the herd shortly before sampling occurs. A false negative test could occur if a PI animal is extremely young or has been recently introduced to the herd. In this scenario, the virus may not have time to disseminate throughout the herd.

Bulk tank RT-PCR

Another method of identifying infected herds is the detection of BVDV RNA in bulk tank milk samples using RT-PCR. RT-PCR is able to identify active BVDV infections because it detects viral RNA. RT-PCR has been shown to be 14.6 times more sensitive than virus isolation and has been proven to be a sensitive and economic method for the detection of a single PI animal within a group of several hundred cows.[92] Drew et al. was able to detect one PI cow in a herd of 162.[93]

RT-PCR has an advantage over virus isolation in that the virus does not need to be replicating for RT-PCR to be positive. It is possible that there will be a high prevalence of BVDV antibody carriers in a herd where a PI cow is present.[92] In this situation it is possible that these antibodies may inactivate virus in the bulk tank sample, rendering the virus isolation negative. However, viral RNA will still be detected by RT-PCR.

There are however, several limitations to bulk tank RT-PCR. One disadvantage is that it only screens cows whose milk contributed to the bulk tank on the day it was sampled and is testing is restricted only to the lactating herd. Therefore, bulk tank RT-PCR cannot report if virus is circulating through the herd. The majority of PI cattle are young stock, and few survive to become part of the lactating herd.

Bulk tank BVDV antibody testing

In dairy herds, BVDV can be detected by analyzing bulk tank samples for BVDV antibodies. This is most commonly done using indirect ELISA.[94-96] In this instance,

results are expressed as optical density absorbance values. The calculated absorbance value from the indirect ELISA is closely correlated to the prevalence of antibody positive cows in the lactating herd. In a Swedish study, herds with low absorbance values < 0.20 had a low or zero prevalence of seropositive animals (prevalence range 0-26.5%), whereas herds with absorbance values > 0.8 had a higher prevalence of seropositive animals (87-100%).[94]

Bulk tank ELISA is the foundation for national BVDV control and eradication programs in many European countries including Sweden, Denmark, Finland, Norway, and the Netherlands.[97] It has also been useful in determining the incidence of BVDV infection within herds. The advantages of bulk tank ELISA include: an easy to obtain sample, the ELISA is quick and inexpensive, and the test is closely correlated to the prevalence of BVDV antibody caries in the lactating herd. The disadvantage of this test is that it is only a reflection of herd exposure and cannot identify active infection. More importantly, this method is not practical in North America due to the widespread use of BVDV vaccines.

Herd Screening Strategies

Several strategies for screening herds have been developed and are used practically to identify PI cattle and BVDV infected herds. These strategies are summarized in Table 1.2.

Whole herd screening has been recommended in the past to identify PI cattle in herds believed to have a problem with BVDV. Because these animals represent less than 1% of the cattle population, the best method to detect a PI animal involves testing an

entire herd followed by the testing of every calf born for the nine months proceeding initial testing and removal of PI animals. Any animal testing positive should be isolated and retested in three weeks before being classified as persistently infected. Whole herd testing can be accomplished by detecting virus or viral antigens in infected cattle using serum, skin, or milk samples.

For producers, whole herd testing often is not practical, as it is a major commitment of both time and money. As a result, sub-sampling methods have been developed for herd surveillance. These include testing newborns and replacement animals, sentinel animal antibody surveillance, and continued calf surveillance. Continued calf surveillance involves using one or several of the diagnostics tests described previously (ACE, IHC, RT-PCR) and evaluates the status of a herd through sampling of the current calf crop. Surveillance of the calf crop gives the veterinarian and producer insight into the herd's BVDV status at that specific point in time and also serves as a means to test the dam. If a calf is BVDV test negative, it suggests that her cow is BVDV-free as well. If a calf is BVDV-PI positive, it suggests that the dam is either a PI or has been exposed to BVDV in the past.

In addition, all replacement animals and their subsequent calves should be tested. The most common way for BVDV to be introduced into a herd is through the purchase of new cattle. To prevent these animals from infecting a herd, they should be screened for BVDV and isolated if possible from the herd until those results are available. In addition, all newborn calves should be tested from any purchased pregnant cattle.[83]

Table 1.2 BVDV Testing Strategies

Strategy	Type	Test	Description
Whole Herd Screening		Pooled RT-PCR Test	Pooling of skin or whole blood/serum samples is an economical way to detect PI animals. Testing can be done on the whole herd including calves. Individual samples are submitted to the laboratory which does the pooling. Testing strategies will identify the individual animals that are infected (PI and acute).
		ACE Test	This test detects BVDV antigen in skin, serum or milk from persistently infected animals. Because it occasionally detects acute infections, animals with positive test results should be retested with viral or antigen detection tests 3 weeks later to confirm PI status prior to culling.
		IHC test	This test detects BVDV antigens from skin samples. IHC requires formalin fixed tissue and has been shown to be valid for detecting PI animals. It also has the advantage of not being adversely affected by the presence of colostral antibodies.
		Virus Isolation	This test uses the mononuclear cells, serum, nasal swabs or tissue samples as the test sample. For acute infections, this is the most reliable sample for a BVDV diagnosis. It can also be used to detect persistently infected animals of any age.

Table 1.2 - continued

Partial Herd Screening	Sentinel Animal Antibody Surveillance	Virus Neutralization	One innovative method of monitoring BVDV circulation within a group of animals is to introduce a sentinel animal to that group and monitor its antibody status utilizing the BVD VN test. The sentinel must be evaluated as a demonstrated non-PI animal and must remain unvaccinated throughout its life within the herd. One example of the use of this animal would be to introduce him to a calf cohort and test him at times critical to the transmission of BVD virus within the cohort. One strategic testing strategy could include three weeks post introduction, at 3 months of age for the cohort, cohort prefreshening, and annually once introduced to the milking herd. This is the only test routinely used to detect and quantify *antibodies* specific for BVDV. The test should be used in unvaccinated animals greater than six months of age.
	Continued PI Calf Surveillance	Pooled RT-PCR IHC ACE	Typically skin samples used. See above for description of tests.
	Replacement Animals	Pooled RT-PCR IHC ACE	Typically skin samples used. See above for description of tests.

Control and Prevention of BVDV

A BVDV control program needs to be both multidimensional and comprehensive with an overall goal to eliminate BVDV from the herd and to maintain BVDV free status for years to come. The key components to an effective control program are biocontainment, biosecurity, and vaccination.

Biocontainment includes the identification and removal of PI cattle from the herd, which is the key component of a BVDV control and prevention program. Once PI cattle are removed from the herd, circulation of BVDV is essentially stopped.

The goal of biosecurity is to reduce the risk of BVDV being introduced to the herd by identifying the risks, understanding the importance of each risk, and managing those risks. Biosecurity can be broken up into three categories: disease screening (diagnostic testing), isolation, and sanitation.[83]

A variety of diagnostic testing strategies are available and were identified previously (Table1.2). Once BVDV has been detected in a herd and all PI cattle have been identified and removed (biocontainment), the next step is to prevent the herd from being reinfected with BVDV. The most common way in which BVDV is introduced into the herd is through the addition of new cattle. Therefore, strict biosecurity is critical for maintaining a virus-free herd. Ideally the herd will remain closed, where no outside cattle are brought into the herd. If not, then all incoming cattle should be tested prior to introduction to the herd. It is also recommended that replacement cows and heifers are open, as pregnant cattle can carry PI calves even if they are not infected. If pregnant animals are purchased, calves should be isolated at birth to determine infection status.

Show cattle should also be isolated for 3-4 weeks following their return to the farm, as these animals are at a high risk of bringing BVDV into the herd.

BVDV typically does not survive outside of its host(s) and is susceptible to common disinfectants. However the virus has been shown to live in manure and has been isolated from manure up to three weeks at temperatures slightly above freezing (41 F).[98] Therefore, sanitation is important, as precautions should be taken to prevent potential BVDV contaminated objects (boots, vehicles, and clothing) from entering the premises.

In addition, other ruminants such as sheep, alpacas, and white-tail deer can become infected and serve as a source of transmission for BVDV.[54-59] Therefore, management strategies to limit or eliminate contact with other ruminants and/or wildlife should be evaluated.

Vaccination plays an important role in the control of BVDV. Although no vaccine is 100% efficacious, vaccination is an option for controlling the spread of virus and reducing the risk of infection in herds.[83] Vaccination also has a role in preventing acute infections that can result in severe disease, as well as reducing the risk of fetal infection. Use of the BVDV vaccine is a sound management practice to reduce the risks associated with BVDV infections. Both killed and modified-live virus (MLV) BVDV vaccines are available. In general, MLV vaccines are believed to be more effective. Vaccine attributes are summarized in Table 1.3.[83] Vaccination protocols are summarized in Table 1.4, but all vaccines should be used according to labeled directions.

Table 1.3 BVDV vaccine attributes

Vaccine Type	**Advantages**	**Disadvantages**
Killed	• Safe in all cattle • Individual doses of vaccine can be removed from bottles over time	• Shorter duration of immunity
Modified-live (MLV)	• Rapid response • Can induce immunity with a single dose • Broader protection • Longer duration of immunity	• Can cause abortion • MLV vaccines must be used within 4 hours after reconstitution

Table 1.4 BVDV vaccination strategies

Vaccination of calves to prevent subsequent disease		
Most Reliable	1	Vaccination after four months of age with two doses of modified live vaccine four weeks apart on the farm of origin prior to weaning, transport, and commingling.
	2	Vaccination after four months of age with a single dose of modified live vaccine on the farm of origin prior to weaning, transport, and commingling.
	3	Vaccination after four months of age with two doses of killed vaccine four weeks apart on the farm of origin prior to weaning, transport, and commingling.
	4	Vaccination prior to four months of age with a single dose of modified live vaccine to healthy calves that nursed adequate colostrums
	5	Vaccination after four months of age with a single dose of killed vaccine on the farm of origin prior to weaning, transport, and commingling.
Least Reliable	6	Vaccination prior to four months of age with a single dose of modified live or killed vaccine to healthy calves that nursed adequate colostrum.
Vaccination of heifers and cows to prevent reproductive losses		
Most Reliable	1	Vaccination of heifers with two doses of modified live vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of modified live vaccine prior to breeding or at branding or weaning.
	2	Vaccination of heifers with two doses of modified live vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of killed vaccine at branding or weaning.

Table 1.4 - continued

	3	Vaccination of heifers with one dose of modified live vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of modified live vaccine prior to breeding or at branding or weaning.
	4	Vaccination of heifers with one dose of modified live vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of killed vaccine at branding or weaning.
	5	Vaccination of heifers with two doses of killed vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of killed vaccine prior to breeding or at branding or weaning.
	6	Vaccination of heifers with two doses of killed vaccine at least 30 days before initial breeding, and annual revaccination with a single dose of killed vaccine prior to breeding or at branding or weaning.
	7	Vaccination of heifers with one or two doses of modified live vaccine at least 30 days before initial breeding, without annual revaccination
Least Reliable	8	Vaccination of heifers and cows each year prior to breeding with a single dose of killed virus.

Eradication Programs

Eradication programs have been in place in Europe since the 1990's.[99] Programs have been put in place in Denmark, Sweden, Norway, Finland, Austria, Germany, and Switzerland.[100-108] The success of these programs led to the establishment of an eradication program in the United States. In Michigan, the Upper Peninsula BVDV eradication program is designed to eradicate the virus for a particular region in Michigan.

One of the goals of the eradication program was to develop effective and economical herd testing strategies such as sentinel antibody testing.

In European BVDV eradication programs, bulk tank antibody testing is the main focal point of most eradication programs. In addition, spot testing is employed in beef herds as well as in follow-up testing of dairy herds.[100] Spot testing involves randomly selecting young animals for antibody testing to predict the presence or absence of infection in a herd. This is based on the high probability of seropositivity in groups of animals where PI animals are present.[36] In Europe, serological testing of random calves using ELISA is part of eradication programs in Denmark, Sweden, Norway, and Austria. The eradication programs in these countries do not involve the use of vaccines and calves selected for testing ranged from 6-12 months of age depending on that nation's program.[38,99,100] In addition, eradication programs in Sweden and Austria take into account herd size when selecting sentinel animals. In Sweden, individual serum samples are collected from 5, 8, or 10 young stock at 12 months of age.[100] The number of animals chosen depends on herd size. In Austria, 15% or at least 5 animals from the young stock are tested serologically twice yearly to test for herd BVDV status.[103] In addition, sentinel testing of calves is seen in Germany where an on-going eradication program includes vaccination.[97,100,109]

The success of these national programs suggest that serological testing of young stock is a valid and useful screening method that can be applied to in an eradication program here in the United States.

Conclusion

BVDV is one of the most economically damaging diseases of cattle. Its complex nature and various clinical manifestations have made control and prevention of BVDV a challenging undertaking. Implementation of proper control measures such as biosecurity and a proper vaccination may reduce the incidence and/or severity of BVDV in a herd. The implementation of a proper screening program is also important for controlling BVDV, although it can prove to be costly to the owner. The potential for less expensive follow-up screening programs is something that should be further pursued in these changing economic times. As eradication projects begin in the United States, less expensive, efficacious follow-up screening programs are something that should be further pursued. One such screening program is the use of sentinel testing, as sentinel animals are a representative sample of a herd that serves to determine the disease status of the herd. The success of national screening programs in Europe suggest that serological testing of young stock is a valid and useful screening method that could be employed in the United States in the form of sentinel animal antibody testing.

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CHAPTER 2
SEROLOGICAL EVALUATION OF SENTINEL CALVES IN A BVDV ERADICATION PROGRAM

Abstract

Objectives. To evaluate serology as a tool to detect herd infection with BVDV as part of an eradication program.

Sample Population. Forty-seven cattle management groups from 36 herds in a regional BVDV Eradication Program.

Procedure. Serum samples were obtained from five non-vaccinated sentinel calves ≥6 months old in each management group and VN antibody titers against BVDV genotypes 1 and 2 were determined. A herd was considered positive if two or more sentinel calves had VN antibody titers ≥128 to either genotype. Results were compared to individual animal testing of all available calves by RT-PCR on skin samples.

Results. In one management group, 3 sentinel calves had VN antibody titers ≥128. Three ear notch samples from that herd were positive for BVDV on RT-PCR assay. All other management groups were negative for BVDV. In this study, the herd sensitivity of sentinel serology was 100% (95% CI, 0.05 to 1.0) and herd specificity was 100% (95% CI, 0.90 to 1.0). The κ value for agreement between sentinel serology and RT-PCR was 1.0 (95% CI 1.0 to 1.0).

Conclusion. Sentinel animal serology can be utilized in a BVDV eradication program to provide an accurate and efficient evaluation of herd status. Although the sensitivity and specificity of serologic surveillance were high in this study, the approach used here may not be suitable for other geographical locations and cattle management systems.

Introduction

Control of bovine viral diarrhea virus (BVDV) has been a worldwide challenge for decades. This virus causes substantial economic loss to the cattle industry, ranging from $10-40 million per million calvings.[1-3] Various clinical manifestations can result from infection with BVDV, ranging from subclinical infection to immunosuppression and respiratory disease to abortion and infertility.[4-7] The most well known source for transmission of BVDV are persistently infected (PI) cattle.[1,2,8] Persistent infection occurs when calves are exposed to BVDV *in utero* before the fetal immune system is developed, thus allowing the developing fetus to become immunotolerant to the virus.[5] PI cattle continuously shed large amounts of virus into the environment and are an important source of virus transmission within and between herds.

Because of the clinical and economical importance of BVDV, many European countries have implemented BVDV eradication programs including Denmark, Sweden, Norway, Finland, Austria, Germany and Switzerland.[9-17] In Michigan, the Upper Peninsula BVDV Eradication Program was launched in 2008 and with the goal of eradicating BVDV from a region within the US. This integrated program included education, identifying and eliminating PI's, vaccination and biosecurity.

A key step in controlling and eradicating BVDV from a herd is the ability to identify and remove all PI cattle. Multiple diagnostic tests have been developed to identify PI cattle including virus isolation (VI), antigen capture ELISA (ACE), immunohistochemistry (IHC), and reverse transcriptase polymerase chain reaction (RT-

PCR). Unfortunately, whole herd testing using any of these tests can be a major investment of both time and money for producers. Therefore developing and implementing alternative herd screening tests would be beneficial for identification of infection with BVDV at the herd level before embarking on individual animal testing.

Serological evaluation of small groups of young non-vaccinated calves using virus neutralization (VN) or ELISA has been proposed as a method for identifying herds with PI cattle.[18-22] The premise is that a high prevalence of seropositive calves in a herd is indirect evidence that a PI animal is present.[20] The calves serve as sentinels for virus being shed by a PI herd mate. Calves selected as sentinels should be non-vaccinated and ≥6 months old because colostral antibodies may be present in the serum of calves < 6 months old.

Initial studies in Denmark and Michigan used a hyper geometric probability function for herds with and without PI cattle to calculate the probability of detecting seropositive animals.[18,19] The Danish study evaluated sera using indirect ELISA and assessed the probability of detecting PI animals when at least two out of five sentinel animals were seropositive at 6-18 months of age.[18] Using that seropositive rate, a predicted sensitivity of 97.7% was proposed for detecting herds containing at least one PI animal. In addition the predicted specificity was 95.2%. The Michigan study evaluated sera using VN titers and determined that the probability of a PI animal being present in a herd was 0.99 if three out of five animals had BVDV antibody titers ≥128. The study further calculated the probability of a PI animal being in the herd was < 0.01 if three of the five animals had titers ≤64.[19] In both studies, all cattle had been tested for both BVDV and BVDV antibodies prior to the study.

Use of sentinel animals in herds of unknown BVDV status to detect presence of cattle persistently infected with BVDV also has been reported. In one study, dairy herds were considered likely to contain PI cattle when at least three out of five heifers, 6 to 12 months old had VN antibody titers ≥128.[21] The sensitivity of using VN titers from sentinel calves to detect BVDV was 66% and the specificity was 100%. Another study done in beef cattle evaluated use of 10 non-vaccinated calves per herd.[22] A herd was classified as likely to contain PI cattle when at least three out of ten calves at weaning had antibody titers ≥1000. Employing this measure, the sensitivity for use of VN titers in sentinel beef calves to detect the presence of a PI animal in a herd was 53% and specificity was 80%.

In Europe, serological testing of random calves using ELISA is part of eradication programs in Denmark, Sweden, Norway, Finland and Austria.[9-12,14-18,23] The eradication programs in these countries do not involve the use of vaccines and calves selected for testing range from 6-12 months of age depending on that nation's program. In addition, sentinel testing of calves is used in Germany where an on-going eradication program includes vaccination.[9,24-26]

The objective of the study reported here was to evaluate the application of sentinel serology as a herd monitoring tool to detect herd infection with BVDV as part of an eradication program in the Upper Peninsula of Michigan.

Materials and Methods

Selection of Herds - Herds (n = 33) enrolled in the Michigan Upper Peninsula BVDV Eradication Program that had completed whole herd BVDV testing in 2008 were asked to voluntarily participate and agree to a sentinel testing scheme as well as a retest of the whole herd in 2009. Twenty-nine herds had tested negative for BVDV in 2008 and 4 herds had at least one calf persistently infected with BVDV diagnosed in 2008. Thirty-one of the herds were beef, one was dairy, and one was mixed dairy and beef. An additional 3 herds (beef) volunteered to participate that were having whole herd tests for the first time in 2009. Some (n = 6) of the 36 herds had more than 1 management group, which was defined as groups of animals on the farm that were managed separately from other animals or groups.

Sample Collection - Skin samples were collected from the ear of calves shortly after birth or at weaning by use of 5/16 ear notcher and then placed in individually labeled bags. These samples were placed on ice or in a refrigerator for storage before being submitted within one week of collection to the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH). The ear notch samples were obtained over the course of 2009. Serum samples were obtained from five non-vaccinated calves ≥6 months old in each herd or, in the case of herds with multiple management groups, each management group within a herd. Collection of serum was done from late summer through late fall of 2009. Samples were placed on ice or in a refrigerator prior to being submitted to DCPAH within one week of collection.

Herd Infection Definition - A herd was considered to be infected with BVDV based on the following criteria:

1. If 2 of 5 calves in any management group had VN antibody titers ≥128 for either genotype 1 or 2 BVDV. Conversely, if 4 of 5 calves in all management groups had VN titers ≤ 64 to both genotypes 1 and 2 BVDV, the herd was considered negative for BVDV.
2. A herd was considered positive if any animal in any management group was found to be positive for BVDV by RT-PCR assay on skin samples.

RT-PCR - Skin samples were analyzed at DCPAH using their standard pooled (n ≤10 ear notches per pool) RT-PCR assay, a hydrolysis probe-based real-time, RT-PCR assay with an internal RNA control to monitor inhibition of the reaction was used to detect RNA from BVDV. The BVDV-specific primers and probe were designed from conserved sequences within the 5' UTR. The assay amplifies both BVDV genotypes 1 and 2 based upon testing of a validation panel provided by Dr. J. Ridpath (NADC, Ames, IA).[27] The detection limit of the assay is 0.8 $TCID_{50}$.[28] If a pool of ear notches was positive, individual samples contained in the pool were confirmed by fluorescent antibody testing of fresh frozen sections of tissue. Any animal positive on initial testing was retested a minimum of 14 days later to confirm persistence of the virus.

Virus Neutralization - Serum samples were tested for VN antibodies against both genotypes 1 and 2 BVDV using standard microtitration assay procedures. Cytopathic Singer strain was used as the genotype 1 BVDV reference strain and cytopathic 125C

strain as the genotype 2 BVDV reference strain. The VN test was conducted using bovine turbinate cells that were free of adventitious BVDV.[29-31] The fetal bovine serum supplement for cell culture medium were also free of live adventitious BVDV, RNA from BVDV, and antibody against BVDV. Serial 2-fold dilutions ranging from 1:4 to 1:4,096 were made for each sample of serum. Antibody titer was considered to be the highest serum dilution at which the cytopathic effect of the BVDV reference strain was completely inhibited.

Data Analysis - Information collected included number of management groups, herd history, and ear notch results. Data was described using descriptive statistics. The sensitivity and specificity of the sentinel calf testing strategy was determined using results of the RT-PCR assay on ear notches as the gold standard. Correlation between the RT-PCR assay and sentinel animal serology as herd based tests was calculated using the κ test statistic.

Results

Herds - Thirty-six herds consisting of forty-seven management groups were included in the study. Ear notch samples were collected from 2,206 cattle during 2009. A total of 395 serum samples were collected in the summer and fall of 2009. Serum samples were collected from calves that were not vaccinated and at least 6 months old at the time of sampling. Description data and results from the herds tested are summarized in Table 2.1. Ear notches from three calves from one management group were positive for BVDV.

Those calves were confirmed to be persistently infected with BVDV on follow-up testing. The same management group was also positive for BVDV based on VN titers of $\geq$ 128 from 3 of 5 sentinel calves for BVDV genotype 1 and 2. The other forty-six management groups tested did not contain PI cattle based on either RT-PCR or VN results.

Analysis of Serologic Data - VN results from non-vaccinated sentinel calves from each management group were compared with the results for herd testing using RT-PCR. The herd sensitivity for VN testing was 100% (95% CI, 0.05 to 1.0) and herd specificity was 100% (95% CI, 0.90 to 1.0). The κ value for agreement between the two tests was 1.0 (95% CI 1.0 to 1.0). To determine the influence of changing the VN cutoff value or the number of calves above or below the VN cutoff value on the accuracy of this strategy, results of serologic testing from each management group were compared with various cutoff values for number of positive calves and minimum antibody concentration. As the number of positive calves and minimum antibody concentration were lowered, the diagnostic specificity of the test decreased (Table 2.2). The sensitivity remained unchanged throughout.

Discussion

The results from this study support and extend earlier studies which found sentinel serologic testing useful for screening herds for infection with BVDV.[19,21,22] In the current study, serological evaluation of 5 non-vaccinated calves per management

group was an accurate herd screening method for predicting the presence of PI cattle in a herd. There was 100% correlation between detection of PI cattle in a herd using RT-PCR on ear notches and the predicted presence of PI cattle in a herd using sentinel serology.

In this study, when a management group had a positive result from sentinel serology using pre established cutoff criteria, a PI was present in the herd and a negative result correlated with no PI's in the management groups. However, since only one management group was positive in the study, the sensitivity cannot be accurately assessed. Previous studies have reported lower sensitivities than that reported here.[21,22] Pillars and Grooms found a sensitivity of 66% in dairy herds when using a diagnostic criteria of 3 of 5 calves with VN antibody titers ≥128.[21] If they had changed their diagnostic criteria to 2 of 5 calves, the sensitivity would have improved to 83%.[32] In that study the diagnostic cutoff criteria was selected based on cost. It was determined that the cost of extensive testing associated with a false positive result was of more concern than the cost of a false negative result. Changing the diagnostic criteria used to define a positive herd would alter the sensitivity and specificity of the test. Sensitivity can be improved by decreasing the VN antibody titer that defines an individual animal as positive. Sensitivity can also be improved by decreasing the number of sentinel calves that have antibody titers above a designated cutoff. Since the goal of surveillance in an eradication program is to identify any and all potentially positive herds, a premium is placed on sensitivity. In this study, as well as in the study done by Pillars and Grooms, decreasing the number of calves which needed to have a minimum titer of ≥128 from 3 to 2 increased sensitivity but did not affect specificity.

Reasons for lowered specificity of sentinel serology discussed in previous studies include vaccination of sentinel calves, persistence of passively acquired antibodies, and acute infections introduced by breakdowns in biosecurity. To address these potential problems, the target population for the study reported here were unvaccinated calves that were 6 to 12 months of age. By testing calves older than 6 months of age, the risk of detecting passively acquired antibodies is decreased significantly as these antibodies are usually undetectable by 6 months of age.[18] In addition, all herds involved in this study were implementing biosecurity measures to reduce the risk of herd infection. This included reducing contact with neighboring cattle farms and screening new cattle for BVDV. These practices help to reduce the risk of herd misclassification

Herd size, multiple management groups and low stocking densities have been cited in previous studies as reasons for lower sensitivity of sentinel serology as a herd based assay for BVDV. Previous studies conducted in dairy herds showed that herd size did not impact the results of serologic testing and suggested that evaluation of five non-vaccinated calves would be effective for herds of varying size as long as the herd was managed as one unit with commingling of cattle of various ages.[18,19,21,23] Since most of the herds in this study (35 of 36 herds) were beef, serologic testing of any and all management groups was done to increase the sensitivity of the test. In addition low stocking density should have been less of a factor, as herds are more contained in the Upper Peninsula of Michigan.

In European BVDV eradication programs, serological testing is part of many eradication programs. This is based on the high probability of seropositivity in groups of animals where PI animals are present.[1] In Europe, serological testing of random calves

using ELISA is part of eradication programs in Denmark, Sweden, Norway, and Austria. The eradication programs in these countries do not involve the use of vaccines and calves selected for testing ranged from 6-12 months of age depending on that nation's program.[8,9,33] In addition, eradication programs in Sweden and Austria take into account herd size when selecting sentinel animals. In Sweden, individual serum samples are collected from 5, 8, or 10 young stock at 12 months of age.[9] The number of animals chosen depends on herd size. In Austria, 15% or at least 5 animals from the young stock are tested serologically twice yearly to test for herd BVDV status.[12] The success of these national programs suggest that serological testing of young stock is a valid and useful screening method that can be applied to in an eradication program here in the United States.

Results from this study provide supportive evidence that serological evaluation of non-vaccinated calves can be utilized as a screening tool for herd infection with BVDV and could be employed as an accurate and economically viable surveillance tool to evaluate herds in a BVDV eradication program. Although the strategy was highly sensitive and specific in the herds assayed for this study, application to herds in different parts of the country and using different management strategies should be evaluated.

Table 2.1 Herd demographic information and results of herd testing using calf ear notching and sentinel serology

				BVDV Status in 2009	
Herd Number	**Number of Management Groups**	**Herd Size**	**Herd History of BVDV**	**Calf Crop Skin RT-PCR**	**Serologic Classification***
1	1	99	Negative in 2008	Negative	Negative
2	1	158	Negative in 2008	Negative	Negative
3	1	105	Negative in 2008	Negative	Negative
4	1	33	Negative in 2008	Negative	Negative
5	1	34	Negative in 2008	Negative	Negative
6	1	24	New in 2009	Positive	Positive
7	1	99	Negative in 2008	Negative	Negative
8	1	17	New in 2009	Negative	Negative
9	1	159	Negative in 2008	Negative	Negative
10	3	190	Negative in 2008	Negative	Negative
11	1	118	Negative in 2008	Negative	Negative
12	1	47	Negative in 2008	Negative	Negative
13	2	303	Positive in 2008	Negative	Negative
14	1	99	Negative in 2008	Negative	Negative
15	5	276	Negative in 2008	Negative	Negative
16	1	100	Positive in 2008	Negative	Negative
17	1	48	Negative in 2008	Negative	Negative
18	1	46	Negative in 2008	Negative	Negative
19	1	19	Negative in 2008	Negative	Negative

Table 2.1 – continued

20	1	207	Negative in 2008	Negative	Negative
21	3	371	Negative in 2008	Negative	Negative
22	1	40	Negative in 2008	Negative	Negative
23	1	39	Negative in 2008	Negative	Negative
24	1	35	Negative in 2008	Negative	Negative
25	1	76	Negative in 2008	Negative	Negative
26	1	22	Negative in 2008	Negative	Negative
27	1	102	Positive in 2008	Negative	Negative
28	1	55	Negative in 2008	Negative	Negative
29	1	24	Negative in 2008	Negative	Negative
30	2	370	New in 2009	Negative	Negative
31	1	90	Negative in 2008	Negative	Negative
32	1	25	Negative in 2008	Negative	Negative
33	1	59	Negative in 2008	Negative	Negative
34	2	249	Positive in 2008	Negative	Negative
35	1	123	Negative in 2008	Negative	Negative
36	1	78	Negative in 2008	Negative	Negative
Total Management Groups	**47**		**Positive herds in 2009**	**1**	**1**

* Based on VN titers, if 2 of 5 calves in any management had titers ≥128 for either type I or II BVDV, the herd was considered infected with BVDV.

Table 2.2 Sensitivity and specificity of serological evaluation of VN antibodies against BVDV for detection of herd infection with BVDV using different diagnostic criteria and when compared with pooled RT-PCR of skin samples.

	BVDV detected from skin samples	
Diagnostic Criteria	**Sensitivity**	**Specificity**
3 of 5 samples ≥128	1.0 (0.05-1.0)	1.0 (0.90-1.0)
2 of 5 samples ≥128	1.0 (0.05-1.0)	1.0 (0.90-1.0)
1 of 5 samples ≥128	1.0 (0.05-1.0)	0.87 (0.73-0.95)
3 of 5 samples ≥64	1.0 (0.05-1.0)	1.0 (0.90-1.0)
2 of 5 samples ≥64	1.0 (0.05-1.0)	0.98 (0.87-1.0)
1 of 5 samples ≥64	1.0 (0.05-1.0)	0.78 (0.63-0.89)
3 of 5 samples ≥32	1.0 (0.05-1.0)	0.96 (0.84-0.99)
2 of 5 samples ≥32	1.0 (0.05-1.0)	0.85 (0.71-0.93)
1 of 5 samples ≥32	1.0 (0.05-1.0)	0.54 (0.39-0.69)

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CHAPTER 3
IMMUNIZATION OF CALVES WITH A MODIFIED-LIVE BOVINE VIRAL DIARRHEA VIRUS VACCINE: EVALUATION OF BOVINE VIRAL DIARRHEA VIRUS IN CLINICAL SAMPLES POST VACCCINATION

Abstract

Objectives. To determine if and for how long vaccine virus can be detected using RT-PCR in skin samples following vaccination with a commercially available modified-live BVDV vaccine

Sample Population. Two different experiments were conducted in this project. Experiment 1 involved 12 BVDV seropositive steer calves, while experiment 2 involved 7 BVDV seronegative heifers.

Procedure. Skin samples were collected in both experiments at days 0 and 3-18 for virus detection using both individual and pooled RT-PCR. In parallel, blood and nasal swabs were collected for virus isolation.

Results. All cattle, regardless of their serological status, were negative for BVDV using individual and pooled RT-PCR on skin samples. Virus was detected by virus isolation in 5 of 7 of the experiment 2 heifers. In addition, all heifers in experiment 2 seroconverted to BVDV.

Conclusion. These findings provide evidence that it is highly unlikely that vaccine virus can be detected in skin by either individual or pooled RT-PCR following vaccination with a commercially available BVDV vaccine.

Introduction

Bovine viral diarrhea virus (BVDV) is an economically important pathogen affecting cattle worldwide. Economic losses associated with BVDV infection have been estimated in the range of $10-40 million per million calvings.[1] A recent study of the economic effects on reproduction and performance in commercial cow herds showed that over a ten year period, calculated an average return to fixed cost of $20.16 less for farms with at least 1 PI in the herd due to reproductive and calf mortality effects.[2] Another recent study has shown that in feedlots, cattle exposed to persistently infected (PI) animals cost the producer an average of $88.26 per animal in performance losses.[3]

Cattle persistently infected with BVDV are the major reservoir for transmission of virus within and between farms. It is estimated that 4% of cow-calf herds and 15% of dairy herds have persistently infected (PI) animals in them.[4] Identification and removal of BVDV PI animals from the herd is a key component for controlling virus transmission. Many different virus detection methods have been used to detect PI's. Currently, the most frequently used methods rely on testing of skin for the presence of BVDV.[5] Methods to detect BVDV in skin include immunohistochemistry (IHC), antigen-capture enzyme-linked immunosorbent assay (ACE), and reverse transcriptase polymerase chain reaction (RT-PCR) assays. Immunohistochemistry was the first test used to detect BVDV antigen in the skin. More recently, ACE and RT-PCR have become the preferred screening tests due to their lower cost, ease of use, adaptation for high volume testing, and analytical sensitivity. RT-PCR has potential for detection of small concentrations of virus, thereby

allowing pooled sampling strategies, which reduce test costs to producers. The high analytical sensitivity of the RT-PCR assay also increases the likelihood of detecting transient infections.

Vaccination programs are a major component of control and prevention strategies for BVDV. While modified-live vaccines (MLV) have become the vaccine of choice for immunization against BVDV, questions have arisen as to whether use of MLV's could lead to false positive results on virus detection assays such as those described above. Several studies have explored the temporal persistence of vaccine virus in various clinical specimens following vaccination with a modified-live virus. BVDV vaccine virus has been shown to remain in the ovaries for up to 12 days after vaccination and a recent study has shown that it can be shed in semen up to 10 days after vaccination.[6,7] Previous studies have shown that when an animal is acutely infected with BVDV, the likelihood of detection of viral antigen in a skin biopsy by IHC or ACE, is very low.[8,9] Other studies have used RT-PCR to test serum and nasal swab samples from calves vaccinated with a modified-live virus vaccine for BVDV. In one study, serum samples from 78% of calves vaccinated were positive for BVDV between 3 and 10 days post-vaccination.[10] However that same study noted that none of the calves sampled had positive results for BVDV using RT-PCR on nasal swabs. While we know from previous studies that BVDV vaccine virus can be detected in blood and certain tissues, to date it has not been found in skin using IHC or ACE.[9,11] It is important to determine if vaccine virus can be detected in the skin because a false positive result for BVDV can lead to more follow-up testing and more costs to the producer. The objective of this study was to determine if and for how

long vaccine virus can be detected using RT-PCR in skin samples following vaccination with a commercially available MLV BVDV vaccine.

Materials and Methods

Two different experiments were conducted in this project using calves known to be free of persistent infection with BVDV.

- *Experiment 1:* Twelve BVDV seropositive steer calves (four to five months of age), were administered a commercially available MLV cytopathic BVDV vaccine[a]
- *Experiment 2:* Seven BVDV seronegative heifers (eleven months to two and a half years of age), were administered a commercially available MLV cytopathic BVDV vaccine[a]

Each group was housed in an isolated pen at least 150 yards from other cattle on the premises.

Clinical Samples

Skin, blood, and nasal swabs were collected from each animal on day 0 (prior to vaccination) and then on days 3 through 14, 16, and 18 post vaccination. Skin samples were collected from the ear using a 5/16 inch ear notcher and then placed in individually labeled bags. Serum samples were collected from all cattle by jugular venipuncture into a serum separator tube. Nasal swabs were collected and placed into tubes containing 1 ml of Dullbecco's modified Eagle medium (DMEM). In experiment 2, whole blood samples

were obtained using EDTA as an anticoagulant. Samples were placed on ice and transported to the laboratory where skin samples were halved and placed in separately labeled bags. Skin, serum and nasal swabs specimens were stored briefly at -20°C until testing. White blood cells were isolated from whole blood samples collected in experiment 2 as follows: after collection, samples were stored at room temperature for one hour and then centrifuged at 1200 rcf for 15 minutes. Following centrifugation, the buffy coat was collected from each sample using individual sterile 1 ml pipettes and placed into labeled 15 ml polypropylene centrifuge tube. The buffy coat with contaminating red blood cells was suspended 3 ml of sterile cell culture grade water for 30 to 45 seconds to lyse the red blood cells, and then 1.5 ml of 2X concentrated physiologic phosphate buffered saline (PBS) was added to the tube. Cells were vortexed and pelleted by centrifugation at 270 rcf for 12 min. A second exposure with 1.5 ml of sterile water, followed by addition of 0.75 ml of 2X concentrated physiologic PBS was done. The cells were pelleted at 270 rcf for 12 min, suspended in 0.5 ml of Bovarnick's solution and frozen at -70°C.

Immediately following vaccination, biological cloning of the viruses contained in the vaccine was performed. This was done in order to compare any strains of BVDV recovered in the samples taken with the strains contained within the vaccine.

Polymerase Chain Reaction Assays - All skin samples submitted to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University. Half of each ear notch was processed for routine diagnostic testing, which was done using pools of homogenate from 10 ear notches for RNA extraction. The RNA was then tested

by a hydrolysis probe-based real-time RT-PCR that targeted the 5′ untranslated (UTR) region of the BVDV genome. An internal RNA control was used to monitor inhibition of the RT-PCR reaction. The detection limit of the assay is 0.8 $TCID_{50}$.[12] Laboratory protocol requires all individual samples in a positive pool of ear notches be retested using fluorescent antibody staining of fresh frozen sections of tissue. All individual samples in a negative pool of ear notches were retested using a gel based RT-PCR targeting the same 5′ UTR region of the viral genome. A positive ear notch would be confirmed using fluorescent antibody staining of fresh frozen sections of tissue.

Virus Isolation - Virus isolation was performed on samples of serum, nasal swabs immersed in 1 ml of transport medium, and white blood cells harvested from whole blood by centrifugation followed by selective lysis of red blood cells. Approximately 200μL of each sample was inoculated onto bovine turbinate (BT) cells in 24-well flat-bottom cell culture plates. Nasal swab transport medium was passed through a 0.45μm syringe filter prior to inoculation. The BT cells were free of adventitious BVDV, as determined by RT-PCR assay. The growth medium for the BT cells was free of adventitious BVDV and antibody against BVDV as determined by virus isolation, RT-PCR assay, and virus neutralization assay against type 1 and type 2 BVDV. The cell monolayers were observed for cytopathic effect (CPE) and any potential BVDV isolations were subpassaged after 5 to 8 days to fresh monolayers of BT cells. After 2 to 5 days, dependent on appearance of CPE, RNA was extracted from infected cells using TRIzol[b] as recommended by the manufacturer. Presence of BVDV was confirmed, and the genotype of the isolate predicted, using RT-PCR assays that targeted the 3′end of genomic region encoding viral

protein NS5B and most of the 3'UTR region of the viral genome [13,14] The RNA from select viral isolates was amplified to obtain nucleic acid sequence that included about 100 bases of the 3' end of the 5' UTR region and all of the genomic region encoding N^{pro} and Capsid viral proteins. To obtain vaccine virus, genotype 1 and genotype 2 BVDV were biologically cloned from reconstituted vaccine. This was done by diluting the vaccine 100 fold in cell culture medium, and then serial 4 fold dilutions were made from the diluted vaccine. The serial dilutions were inoculated onto BT cells that had been seeded into 96 well microtitration plates. Culture fluid was harvested from wells showing CPE typical of BVDV and used to inoculate 24 well plates. After appearance of CPE, RNA was extracted from infected cells and amplified for nucleic acid sequencing.

Genetic Analysis - The samples of amplified nucleic acid were submitted to the Michigan State University Research Technology Support Facility for DNA sequencing. Sequences were aligned and trimmed using software described previously.[15] Phylogenetic analyses were conducted by use of integrated software as previously described.[16]

Virus Neutralization - Serum samples were tested for viral neutralizing (VN) antibodies against genotypes 1a, 1b and 2a BVDV using standard microtitration assay procedures. The cytopathic Singer strain was used as the genotype 1a BVDV reference strain, cytopathic strain TGAC as the genotype 1b reference strain, and cytopathic 125C strain as the genotype 2a BVDV reference strain. The VN test was conducted using bovine turbinate cells that were tested free of adventitious BVDV.[17-19] The fetal bovine serum

supplement for cell culture medium tested negative for live adventitious BVDV, RNA from BVDV, and antibody against BVDV. Serial 2-fold dilutions ranging from 1:4 to 1:4,096 were made for each sample of serum. Antibody titer was considered to be the highest serum dilution at which the cytopathic effect of the BVDV reference strain was completely inhibited.

Results

RT-PCR

All skin samples collected from all vaccinated cattle in both experiments were negative for BVDV at all time points using both pooled RT-PCR and individual sample RT-PCR.

Virus Isolation

In experiment 1, BVDV was not isolated from either serum or nasal swabs. In experiment 2, BVDV was not isolated from nasal swabs, but a total of twenty BVDV isolates were identified from serum and buffy coat samples. Four of seven animals were positive on virus isolation from serum on at least one day (n=11) and five of seven were positive on virus isolation from serum on at least one day (n=9). RT-PCR characterized 18 isolates as BVDV biotype 1 and 2 isolates as BVDV biotype 2. Both BVDV biotype 1 and 2 were isolated from 2 of the positive cattle. Results from experiment 2 are summarized in table 1. A representative sample (n=14) of BVDV isolates from each calf were selected and sequenced to compare to the vaccine strains as well as to look for any variation in virus within the individual animal. Genetic sequencing showed that the

viruses isolated were 99.9% similar to each other (Figure 3.1). In addition the sequenced isolates aligned with the type 1 and type 2 viruses contained within vaccine used in the experiment.

Virus Neutralization

Eleven of 12 calves in experiment 1 had BVDV VN antibodies on day 0 prior to vaccination and the 12th calf seroconverted by day 18. Conversely, all 7 animals in experiment 2 were naïve for BVDV antibodies on day 0, and all 7 had seroconverted to BVDV genotype type 1a and 1b by day 18 and 6 of 7 had converted to BVDV genotype type 2a by day 18 (Table 2).

Biological Cloning/Sequence Analysis

Biologic cloning of the reconstituted vaccine led to 34 isolates being made. Of the 34 isolates, RT-PCR amplification led to 1 isolated being classified as BVDV biotype 1, 25 isolates classified as biotype 2, and 8 were a mix of both biotypes 1 and 2. Genetic sequencing of the type 1 and type 2 specific isolates showed 99.9% alignment with the type 1 and type 2 viruses that were reported to be contained within the vaccine (Pfizer Animal Health, Personal Communication).

A representative sample (n=14) of BVDV isolates from each calf were selected and sequenced to compare to the vaccine strains as well as to look for any variation in virus within the individual animal. Genetic sequencing showed that the viruses isolated were 99.9% similar to each other (Figure 3.1). In addition the sequenced isolates aligned with the type 1 and type 2 viruses contained within vaccine used in the experiment.

Discussion

In this study, all cattle, regardless of their serological status, were negative for BVDV using individual and pooled RT-PCR on skin samples following vaccination with a modified- live BVDV vaccine. These findings indicate that there is a low probability for detection of vaccine virus in ear notch samples by either individual or pooled RT-PCR following vaccination with the commercially available BVDV vaccine used in this study. These findings are similar to previous studies that evaluated skin samples following vaccination using IHC.[8,9,11] In these studies, the authors were unable to detect BVDV in skin samples from animals that were either acutely infected[8,9] or vaccinated with BVDV.[11]

Bovine viral diarrhea virus was not found in any of the samples from calves in experiment 1. This result was not surprising because of the pre-existing VN antibody titers present in these calves. In experiment 2, RT-PCR assays (pooled and individual) did not detect virus in skin samples of vaccinated animals on any of the sample dates. However, virus isolation from serum and buffy coat, and seroconversion of calves provides evidence that the vaccine viruses were replicating in the animals during the testing period. Results from virus isolation of nasal swabs, serum, and buffy coat samples in experiment 2 were similar to previous findings for seronegative cattle.[8,10,20] Reasons why vaccine virus may not have been detected in skin samples include virulence of the vaccine strain and location of virus replication. Modified-live vaccines typically are of reduced virulence and do not replicate to high titers, as compared to virulent field strains

of BVDV. It should be noted that when virus isolation was performed on serum and buffy coats, CPE was often not observed until 5 or more days after cell culture inoculation. This suggests that there were small amount of viable virus in these samples. If a small amount of virus exists in the blood, then the probability chances of detecting virus from a skin sample using any assay including RT-PCR is small. Furthermore the attenuation of the vaccine may diminish viral replication in vivo in the same sites as field strains of BVDV might. Previous studies have shown that cytopathic BVDV can be found in the skin of cattle undergoing mucosal disease[21], however to our knowledge no studies have looked at distribution of modified-live cytopathic vaccine virus in skin.

One limitation of this study was that only one commercially available modified-live BVDV vaccine was used. The findings from this study should not be extended to other modified-live or killed vaccines. In addition, there were no non-vaccinated controls used in these experiments. Non-vaccinated controls would have served to detect extraneous virus exposure during the course of the study. However, this limitation was accounted for by sequencing virus isolated from calves and comparing that sequence back to the vaccine virus used in the study. The fact that all virus detected were identical to the viruses present in the vaccine provides substantial evidence that the virus detected originated from the virus administered.

The results of this study provide evidence that it is unlikely to detect BVDV by RT-PCR on skin samples following vaccination with a commercially available modified-live BVDV vaccine. This study supports findings from other studies that have shown similar results using different assay methods. Veterinarians and producers should feel

confident that positive test results for BVDV on skin samples is unlikely to be caused by vaccination virus following administration of a modified-live virus vaccine.

Sources and Manufacturers

a. Bovi-Shield Gold 5, Pfizer Animal Health, New York, NY

b. TRIzole Reagent, Invitrogen Co., Carlsbad, Calif.

Table 3.1 BVDV biotypes isolated in Experiment 2 from Day 7-14

	Animal ID													
	Serum							Buffy Coat						
Day	**82**	**83**	**84**	**86**	**88**	**89**	**91**	**82**	**83**	**84**	**86**	**88**	**89**	**91**
7	-	-	-	1a	-	-	-	-	-	-	1a	1a	-	-
8	-	-	-	1a	-	-	-	-	-	-	1a	2a	-	-
9	-	-	-	1a	-	-	-	-	-	-	-	-	-	-
10	-	-	-	1a	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	1a	1a	-	-	-	-	-	-	1a	-
12	-	-	1a	1a	-	1a	-	-	-	1a	-	-	2a	1a
13	-	-	-	1a	-	1a	-	-	-	-	1a	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.2 Virus Neutralization results at day 0 and day 18 post vaccination with a commercial modified-live BVDV vaccine (day 0/day18).

	VN titers d 0/18		
Animal ID	**Singer 1a**	**TGAC 1b**	**125C 2a**
Experiment 1			
40W	256/32	16/16	64/32
41W	512/512	128/128	256/256
46W	32/32	16/8	32/16
50W	4096/2048	1024/512	128/128
904	4/2048	4/128	4/256
906	32/64	4/4	128/64
907	16/128	4/16	16/8
909	32/128	8/32	4/2048
911	2048/512	128/128	256/64
912	128/128	32/16	8/16
914	1024/2048	128/64	1024/256
937	16/16	4/4	8/4
Experiment 2			
82	<4/256	<4/32	<4/4
83	<4/512	<4/64	<4/128
84	<4/512	<4/128	<4/32
86	<4/64	<4/128	<4/4
88	4/64	<4/16	<4/4
89	<4/32	<4/64	<4/64
91	<4/16	<4/<4	<4/4

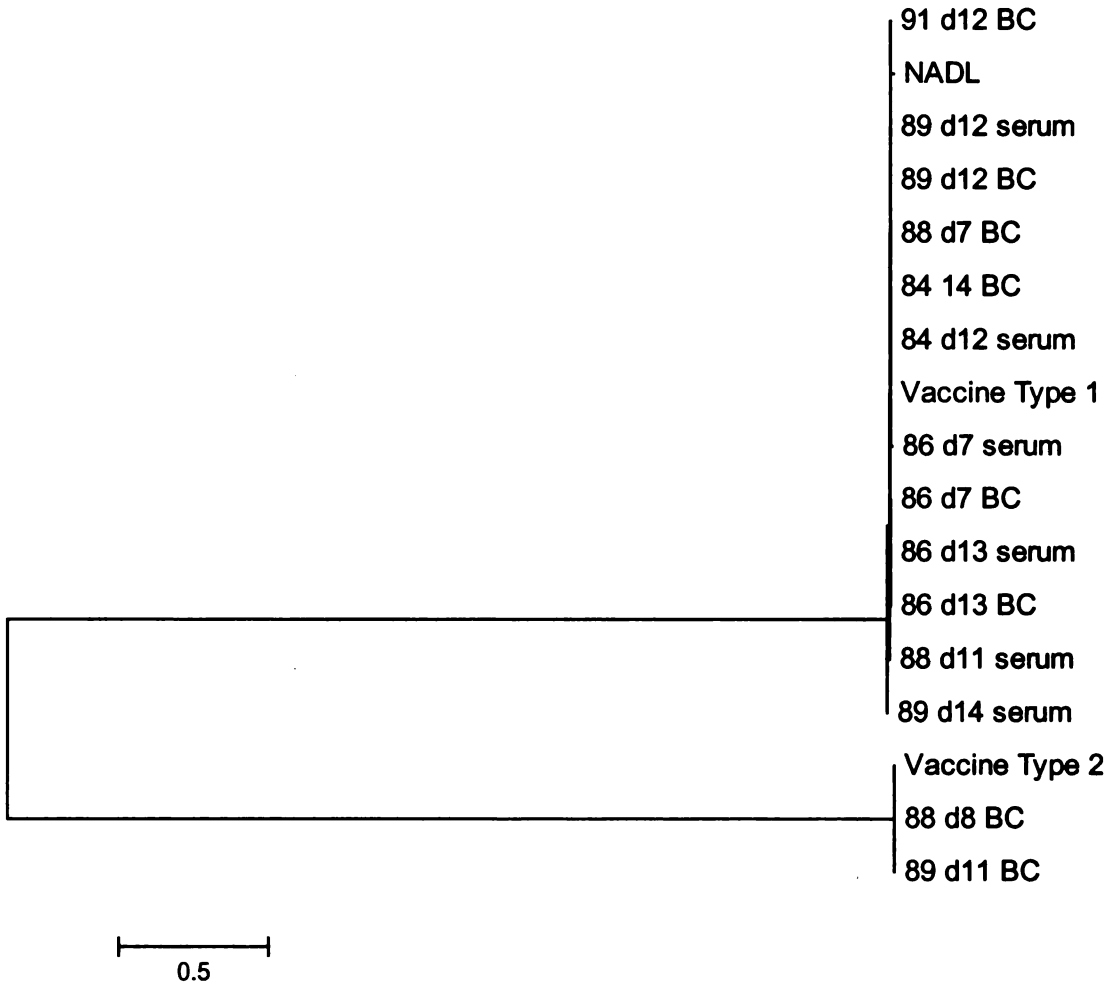

Figure 3.1 Phylogenetic relationships of 14 BVDV isolates to genotype 1 (Vaccine Type 1) and genotype 2 (Vaccine Type 2) BVDV vaccine used in experiment 2. The neighbor-joining method was used to generate this phylogenetic tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by use of the Jukes-Cantor method and are in the units of the number of base substitutions per site.

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SUMMARY

Sentinel serology is a useful method for detecting the presence of BVDV in cattle herds. This strategy appears to be an effective and economically viable secondary test for follow-up testing in herds that have completed whole herd testing previously. In the case of an eradication program, it is important to develop inexpensive means of surveillance testing that allows for on-going herd monitoring. In a voluntary eradication program, having a cost-effective test available will aid producers in continuing to monitor their herd without a significant financial burden. If a mandatory government program is in place, sentinel testing also fits in as an excellent surveillance tool that is easy to implement and economically feasible, especially when financial resources are limited.

In addition, at the herd level, knowing if a herd has been re-infected with BVDV will allow veterinarians and producers to modify biocontainment, biosecurity, and vaccination protocols. Likewise, knowing that a herd is still BVDV-free, confirms that any control protocols implemented appear to be working. It may also allow for herd owners to market their animals as BVDV free. From a regional standpoint, it will allow veterinarians to isolate and research any BVDV positive herds to determine how the disease was re-introduced to the herd so that spread of the disease can be controlled and prevented. The ability to identify where the disease came from will allow veterinarians and producers alike to take the proper precautions to prevent re-introduction of the virus into additional herds.

In the face of a positive BVDV results, producers could be faced with costs associated with further individual or herd testing or more significantly, culling of suspected PI cattle. Therefore, false positive test results can be very costly to the

producer. As newer and more sensitive diagnostic tests are developed, it is important to conduct studies such as the one reported in this thesis, in order to confidently interpret the results. BVDV vaccine virus could not be detected in skin following vaccination with a modified live BVDV vaccine. When coupled with findings of previous studies, this suggests that it is unlikely to detect BVDV by RT-PCR on skin samples following vaccination. Thus, veterinarians should be able to confidently tell producers that recent vaccination of cattle with a MLV BVDV vaccine should not lead to false positive results.

The vaccine study ties into the eradication of BVDV from a specific region in the sense that by knowing that it is highly unlikely to find vaccine virus in the skin samples of cattle. Therefore veterinarians and producers should feel confident that positive test results for BVDV on skin samples is unlikely to be caused by vaccination virus following administration of a modified-live virus vaccine. Positive BVDV results from any form of testing should serve as a red flag that BVDV exists within that herd and steps should be taken to isolate and eliminate any animals with the virus.

The Future

In a perfect world, the sentinel serology study could be repeated with a larger sample size and using herds with both known and unknown BVDV status. Having a greater number of herds with BVDV present would allow for sensitivity and specificity to be more accurately measured. However it should be stressed that the goal of this particular study was to evaluate the sentinel serology in an eradication project, not to determine the sensitivity and specificity of the test. In addition, since this test is being evaluated as a follow-up surveillance tool, it would be interesting to conduct this study

over several years. By doing so, producers would be able to see if their biosecurity and vaccination protocols have prevented the re-introduction of BVDV into their herds, as well as determining if sentinel serology works as a regional surveillance tool. Of course it should be noted that there are always exceptions to the rule. Many herds exist where sentinel serology may prove to be impractical. One such example is any herd where vaccination programs begin within the first weeks or months of a calf's life. In herds such as this, sentinel serology would be less practical and other screening modalities would need to be explored. One way to overcome this problem would be to leave 5 calves unvaccinated until sentinel testing occurs or by placing a separate sentinel species, such as sheep, within the herd.

Regarding the vaccination study, to the most important way to build upon this study would be to evaluate different vaccines on the market. In addition to other modified-live cytopathic vaccines, evaluation of modified-live non-cytopathic vaccines, as well as killed vaccines would be of value. By doing this, one would be able to more confidently state that vaccine virus cannot be detected in skin samples using RT-PCR following vaccination.

In summary, our research has advanced cattle health and well-being by further demonstrating the usefulness of sentinel serology as a tool for controlling and preventing BVDV in cattle, specifically in its application in a BVDV eradication program in North America. Furthermore, we have provided strong evidence that modified live BVDV vaccines are unlikely to cause false positive results on common BVDV detection assays, thus simplifying the interpretation of these test results.