

MANAGEMENT OF BOVINE VIRAL DIARRHEA VIRUS IN BEEF HERDS

by

JASON S. NICKELL, DVM

B.S., University of Missouri-Columbia, 1999

D.V.M, University of Missouri-Columbia College of Veterinary Medicine, 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology  
College of Veterinary Medicine

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2010

## **Abstract**

Bovine viral diarrhea virus (BVDV) is an endemic pathogen in the U.S. cow herd. The virus can cross the placental barrier and infect the unborn fetus. If infection occurs between 45 – 125 days of gestation, persistent infection (PI) in the unborn fetus is likely. Upon parturition, the PI calf is a lifelong shedder of BVDV significantly elevating the risk of viral exposure to non-PI cattle.

Despite reports of significant production loss, many BVDV infections are subclinical and in some cases inconsequential. Our data has highlighted various factors potentially causing disparity in clinical outcomes following BVDV exposure including: variation of BVDV serum concentration among PI cattle which may influence the quantity of virus shed into the environment, preexisting BVDV immune (i.e. antibody) status among non-PI cattle, and the degree of stress experienced by non-PI cattle all may influence the susceptibility of disease. Additionally, cattle transiently infected (TI) with BVDV may temporarily shed BVDV thereby offering another source of exposure to non-PI cattle.

Programs focusing on BVDV control and prevention consist of diagnostic tests to identify PI cattle, BVDV vaccines to reduce fetal infection and increase herd immunity, and biosecurity programs intended to prevent BVDV exposure to the resident herd. Survey work performed in Montana suggest that educating beef producers with regard to BVDV has significantly increased the implementation of these tools in order to reduce the risk of introducing BVDV to their resident herd.

Despite the risk of production loss, the economic benefit of instituting whole-herd BVDV tests may vary due to herd prevalence. By utilizing Monte Carlo simulation, the current BVDV herd prevalence within the U.S. does not economically justify a nationwide BVDV eradication campaign. However, known BVDV positive herds and herds with an elevated likelihood (47%) of being BVDV positive displayed a positive economic outcome when whole-herd BVDV testing strategies were implemented across herd sizes of 50, 100, and 500 cows. The value of testing various testing modalities was dependent upon herd prevalence and herd size. These data suggest that veterinarians must critically evaluate the value of implementing whole herd testing protocols in U.S. beef herds

MANAGEMENT OF BOVINE VIRAL DIARRHEA VIRUS IN BEEF HERDS

by

JASON NICKELL, DVM

B.S., University of Missouri-Columbia, 1999  
D.V.M., University of Missouri-Columbia College of Veterinary Medicine, 2003

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology  
College of Veterinary Medicine

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2010

Approved by:

Co-Major Professor  
Robert L. Larson

Approved by:

Co-Major Professor  
Bradley J. White

# **Copyright**

Jason Nickell, DVM

2010

## **Abstract**

Bovine viral diarrhea virus (BVDV) is an endemic pathogen in the U.S. cow herd. The virus can cross the placental barrier and infect the unborn fetus. If infection occurs between 45 – 125 days of gestation, persistent infection (PI) in the unborn fetus is likely. Upon parturition, the PI calf is a lifelong shedder of BVDV significantly elevating the risk of viral exposure to non-PI cattle.

Despite reports of significant production loss, many BVDV infections are subclinical and possibly inconsequential. Our data has highlighted various factors potentially causing disparity in clinical outcomes following BVDV exposure including: variation of BVDV serum concentration among PI cattle which may influence the quantity of virus shed into the environment, preexisting BVDV immune (i.e. antibody) status among non-PI cattle, and the degree of stress experienced by non-PI cattle may all influence the susceptibility of disease. Additionally, cattle transiently infected (TI) with BVDV may temporarily shed BVDV thereby offering another source of exposure to non-PI cattle.

Programs focusing on BVDV control and prevention consist of diagnostic tests to identify PI cattle, BVDV vaccines to reduce fetal infection and increase herd immunity, and biosecurity programs intended prevent BVDV exposure to the resident herd. Survey work completed in Montana suggests that knowledge of BVDV among beef producers has significantly increased the implementation of these tools in order to reduce the risk of introducing BVDV to their resident herd.

Despite the risk of production loss, the economic benefit of instituting whole-herd BVDV tests may vary due to herd prevalence. By utilizing Monte Carlo simulation, the current BVDV herd prevalence among cow-calf herds within the U.S. does not economically justify a nationwide BVDV eradication campaign based upon whole-herd testing. However, known BVDV positive herds and herds with an elevated likelihood (0.47) of being BVDV positive possessed a positive economic outcome when whole-herd BVDV testing strategies were implemented across herd sizes of 50, 100, and 500 cows. The value of various testing modalities was dependent upon herd prevalence and herd size. These data suggest that veterinarians must critically evaluate the value of implementing whole herd testing protocols in U.S. beef herds.

## Table of Contents

List of Figures .....	ix
List of Tables .....	x
Acknowledgements .....	xii
Preface .....	xiii
CHAPTER 1 - The Effects of Bovine Viral Diarrhea Virus in Beef Production Systems .....	1
Overview .....	1
History and Background of BVDV .....	1
The Role of BVDV in Bovine Reproduction .....	3
The Manifestation and Clinical Implications of Cattle Persistently Infected with BVDV .....	5
Genetic Variability of BVDV .....	8
The Role of BVDV in the Bovine Respiratory Disease Complex .....	9
Diagnosis and Control of BVDV .....	11
BVDV Diagnostic Tests .....	11
BVDV Vaccination .....	12
Biosecurity .....	14
BVDV Risk Analysis .....	15
Conclusion .....	15
CHAPTER 2 - Variation in Viremic Concentration among Cattle Persistently Infected with Bovine Viral Diarrhea Virus .....	17
Introduction .....	17
Materials and Methods .....	18
Study 1 .....	18
Study 2 .....	18
Study 3 .....	19
Virus Isolation .....	19
Statistics .....	20
Results .....	21
Study 1 .....	21

Study 2 .....	21
Study 3 .....	21
Discussion.....	22
Sources and Manufacturers.....	25
CHAPTER 3 - Evaluation of health and performance outcomes in stocker calves following the administration of a commercial vaccine containing a bovine viral diarrhea virus contaminant .....	
	29
Introduction.....	29
Materials and Methods.....	30
Fall 2007 Trial.....	30
Spring 2008 Trial .....	31
Serology .....	32
Real-Time Polymerase Chain Reaction .....	32
RNA Sequencing .....	33
Statistical Analysis.....	33
Results.....	34
Fall 2007 Trial.....	34
Spring Trial 2008 .....	35
Discussion.....	36
Conclusion .....	40
CHAPTER 4 - Evidence of viral transmission and nasal shedding among beef calves exposed to a calf persistently infected with bovine viral diarrhea virus.....	
	45
Introduction.....	45
Materials and Methods.....	46
Serology .....	48
Virus Isolation.....	49
Real-time Reverse Transcriptase Polymerase Chain Reaction.....	49
Statistical Analysis.....	50
Results.....	51
Discussion.....	52
Sources and Manufacturers.....	58

CHAPTER 5 - Bovine Viral Diarrhea Virus (BVDV) Status in cow-calf herds, its association with biosecurity practices, and the association of these practices with self-perceived understanding of BVDV among Montana beef producers .....	61
Introduction.....	61
Materials and Methods.....	62
Sample Population .....	62
Survey Instrument.....	63
Survey implementation .....	63
Statistical analysis.....	63
Results.....	64
Descriptive BVDV outcomes among project participants.....	64
Survey outcomes in the present study.....	64
Association of survey outcomes and herd BVDV status .....	66
Association of perceived understanding of BVDV and herd biosecurity practices.....	67
Discussion.....	67
Sources and Manufacturers.....	72
CHAPTER 6 - Quantification of the Value of Implementing Whole Herd Testing Strategies in U.S. Beef Herds .....	76
Introduction.....	76
Materials and Methods.....	77
Model structure .....	77
Sensitivity Analysis .....	82
Application of the Model .....	82
Results.....	82
Application of the model .....	82
Sensitivity Analysis .....	83
Discussion.....	84
Sources and Manufacturers.....	89
CHAPTER 7 - Dissertation Conclusion .....	99
CHAPTER 8 - Bibliography.....	100



## List of Figures

Figure 2.1: Distribution of BVDV serum concentrations at one point in time among 33 PI animals composing Study 1. ....	26
Figure 2.2: Individual serum BVDV concentrations among PI calves (n=6) in Study 2 measured weekly by VI for four weeks. Note that calf # 4979 is missing data in Week 1.....	27
Figure 2.3: Individual serum BVDV concentration for two PI calves at each sample collection time point across a 45 day study period.....	28
Figure 4.1: Individual serum BVDV concentrations for the PI calves in studies one and two at each sample collection time point across a 21 day study period. The cell-culture infectious dose <sub>50</sub> /ml (CCID-50) values (y-axis) reflect serum viral concentration in log <sub>10</sub> format (e.g. log <sub>10</sub> 3 = 10 <sup>3</sup> ).....	60
Figure 5.1: Within-herd prevalence of PI cattle among all BVDV positive herds (n=38) voluntarily enrolled in the Montana BVD-PI Biosecurity Program from 2006 – 2009. ....	73
Figure 6.1: Graphical display of the value of implementing whole-herd BVDV tests for each of the 12 BVDV testing strategies in a 100 cow beef herd at each of the three BVDV herd prevalences evaluated in the present study. Error bars denote 95% confidence intervals...	97

## List of Tables

Table 3.1: Model-adjusted <sup>1</sup> means and 95% confidence intervals for health measures (morbidity, repull, mortality, and days on feed (DOF) at first pull) and average daily gain (ADG) by BVDV status (by PCR) at revaccination for the Fall 2007 study. ....	41
Table 3.2: Model-adjusted probability and 95% confidence intervals measuring the risk of individual cattle testing positive for BVDV on day 10 based on BVDV antibody status <sup>1</sup> at arrival. ....	42
Table 3.3: Risk <sup>1</sup> and 95% confidence intervals for testing positive for BVDV by genetic sequence on days 7, 14, and 21, post-vaccination of cattle in Spring 2008 study (n=22). ....	43
Table 3.4: Risk <sup>1</sup> and 95% confidence intervals for developing titers to BVDV genotypes 1 and 2 on days 7, 14, and 21, post-vaccination of cattle in Spring 2008 study (n=22). ....	44
Table 4.1: Individual animal outcomes among the non-persistently infected calf population across studies one (n=12) and two (n=15). Serum and nasal swabs were collected every other day and every fourth day (including day 21), respectively. Cumulative serum and nasal swab PCR status reflects the individual animal status at day 21 (post PI introduction). ....	59
Table 5.1: Descriptive statistics of continuous variables among the respondent population (n=230) that provided answers to the following questions. ....	74
Table 5.2: Descriptive statistics of categorical variables among the respondent population (n=230) that provided answers to the following survey questions. ....	75
Table 6.1: BVDV testing modalities evaluated in the present study. ....	90
Table 6.2: Input distributions for numerous baseline herd-level factors used in the model. ....	91
Table 6.3: Input distributions describing the additional health effects inflicted by BVDV. ....	92
Table 6.4: Input distributions regarding laboratory fees for individual BVDV diagnostic tests. ....	93
Table 6.5: Input distributions for diagnostic tests listed in Table 3. ....	94
Table 6.6: Model-adjusted <sup>1</sup> means (and 95% confidence intervals) of the value of performing whole herd BVDV tests in U.S. cow-calf herds at herd sizes of 50, 100, and 500 head. For both independent factors (individual herd BVDV status and herd BVDV prevalence), superscripts within each column denote statistical differences at the 0.05 level. ....	95

Table 6.7: Model-adjusted<sup>1</sup> means for BVDV testing strategy by herd prevalence interaction within herd sizes of 50, 100, and 500 head. Superscripts within each column denote statistical differences at the 0.05 level. .... 96

Table 6.8: Sensitivity analysis for Test 2 (initial test: IHC, confirmatory test: IHC) and Test 3 (initial test: ACE, confirmatory test: none) at each respective herd size and herd BVDV prevalence. Within each column, the top five contributors to variance are displayed. The values reflect the individual percentage of variation of the outcome variable (cumulative value of testing for BVDV across a two year timeframe)..... 98

## **Acknowledgements**

I would like to thank my PhD committee for their time, commitment, and perseverance during my tenure as a graduate student at Kansas State University. Their professionalism mixed with a sense of friendship provided ongoing momentum throughout my program. From day one, I have continued to learn and be challenged while developing a method of critical thinking that extends beyond individual animal medicine or traditional herd health practices. I would especially like to extend a special acknowledgement to Dr. Bob Larson. Thanks Dr. Larson for keeping the door open long enough for me to get to K-state. Although it may seem insignificant now, that gesture will go a long way in my book. I hope to have the chance to work with all of you in the future and build upon the relationships that were made over these last three years.

I would like to extend a huge thank-you to my fellow graduate student colleagues who made every day a little easier simply by being present. Whether it was a formal class setting, research projects, or a night out in Aggieville, there was always the recognition that although a lot of work needed to be done, there was still room for a little enjoyment. There is something about experiencing a significant life-changing experience with other individuals that provides a sense of loyalty and dedication. I hope that you all know that you can call me for anything at any time and that my door is always open.

My greatest acknowledgement, however, belongs to my family. Stacey, you are the cornerstone of our family, the love of my life, and have continually supported me through not only this graduate program but in every facet of our married life. I am confident that I would not have possessed the strength to take on the challenges of this program without your love and support. In fact, I can't imagine going through this life without you by my side. To my kids, Brooke and William, you two have filled an emptiness that I did not know existed. You both provide me with more joy than I could ever describe and I love you more than you will ever know. The greatest part of my day is opening the door of our house in the evening and hearing the voices coming from the other side. I love you all and am so proud that we are a family.

## Preface

The body of work in the ensuing chapters provides a brief overview of the complexity of BVDV itself and the decisions to institute control and prevention strategies. This intricacy is driven not by the inability to diagnose or prevent the disease but by the question of whether or not it is economically valid to do so. Given prior data displaying the wide variation in clinical and economical outcomes, the honest answer to the question of economical justifiability of implementing diagnostic and control plans is “it depends”. The ambiguity of this answer is frustrating to many (especially diagnosticians) but is the reality of biology and the world in which the practicing veterinarian resides. Therefore, the economic effectiveness of a BVDV control plan solely depends upon the risk of introduction (i.e. prevalence of disease), the likelihood that BVDV will cause disease (i.e. pathogenicity) if infected, and the impact that disease may have on health and production. The veterinary literature provides several different clinical and economic outcomes (spanning from economic devastation to inconsequential findings) when cattle are exposed to BVDV. The obvious question that arises from these observations is what is the cause (or more likely “causes”) of this variation? In our own work, we have observed this variability first-hand (Chapters 3 and 4), evaluated one potential source of variation (Chapter 2), assessed numerous management practices and their association with herd BVDV status (Chapter 5), and determined the economic feasibility of implementing a BVDV diagnostic testing protocol based upon the intended testing strategy, BVDV herd prevalence, and herd size (Chapter 6). Given the findings in the following chapters, individual points of view regarding BVDV control (e.g. federal animal health regulators, diagnosticians, and private practitioners) will likely differ based upon clinical background and field experience with BVDV. Nonetheless, all things considered, decisions regarding BVDV control and prevention strategies must be made in accordance with what program (if any) is most economically effective and possesses the greatest likelihood of compliance at the farm level. All in all, failing to evaluate all available data in regards to the economic justification of implementing a BVDV control strategy, veterinarians are at risk of committing a very costly mistake to the producer.

# CHAPTER 1 - The Effects of Bovine Viral Diarrhea Virus in Beef Production Systems

## Overview

Upon first being described in 1946, bovine viral diarrhea virus (BVDV) has enthralled and perplexed the veterinary medical community worldwide. The fascination with this pathogen is likely attributed to the biology and subsequent complexity of the virus, the potential to manifest in various clinical syndromes, and the uniqueness of the production of immunotolerant and persistently infected (PI) cattle which serve as an inapparent carrier and source of virus to healthy animals.

Despite the negative clinical implications historically associated with BVDV, clinical outcomes can be highly variable. Prior research and anecdotal reports suggest that BVDV can manifest as both a highly detrimental pathogen to any and all bovine production systems or be an inconsequential finding.<sup>1-13</sup> These observations are intriguing and fuel continual debate regarding the proper avenues and the scale of monetary resources that should be directed towards prevention and control. The objective of this chapter is to provide a broad overview of BVDV, provide examples of variation in clinical outcomes, describe the prevalence of certain management practices potentially associated with BVDV introduction, and address the need of assessing the economic variability in the implementation of current PI animal testing protocols.

## History and Background of BVDV

The clinical effects of BVDV were first described as a case series during the 1946 meeting of the New York State Veterinary Medical Association.<sup>1</sup> At the time of that meeting, the disease had been observed in six dairy herds; however, the etiology of this novel syndrome had not yet been discovered. The original clinical description indicated that this syndrome was primarily composed of severe gastro-enteritis with subsequent persistent diarrhea.<sup>1</sup> Across all six herds, the risk of morbidity and mortality was observed to range from 33% – 88% and 4% – 8%, respectively. Additional clinical signs early in the course of disease included depression, anorexia, and mild diarrhea with a slightly elevated rectal temperature. Within two days after the onset of diarrhea, affected cattle were observed to develop ulcerations on the muzzle, in the mouth, and in the nose. Muco-purulent nasal discharge was evident while some diseased cattle

1 displayed a cough throughout the course of the disease process coupled with tachycardia and  
2 tachypnea. Milk production was shown to be severely reduced and complete blood counts  
3 revealed moderate to severe leukopenia. Following acute disease onset, abortions were observed  
4 in five of the six herds while some cows were noted as to have aborted without evidence of  
5 clinical disease. Symptomatic treatment was provided which included antimicrobial therapy and  
6 blood transfusions. However, the authors noted that all treatment modalities (including large  
7 doses of penicillin) did not appear to provide clinical benefit.<sup>1</sup>

8 Experimentally, the authors observed that the disease was transmissible by fecal-oral  
9 exposure, by subcutaneously injecting naïve cattle with blood from infected individuals, and by  
10 oral exposure to splenic emulsion from diseased cattle (euthanized during the height of the  
11 disease process).<sup>1</sup> Naïve heifers exposed to various inoculants were shown to develop a fever  
12 seven to nine days post-exposure lasting in duration of three to six days. Diarrhea was observed  
13 to ensue soon thereafter lasting three to five days while severely compromising the hydration  
14 capability of the individual animal. One to three days after the onset of diarrhea, oral ulceration  
15 was observed thereby worsening the current anorexia and dehydration status. Ulcerations were  
16 noted throughout the oral cavity, naso-pharynx, larynx, and esophagus. Lower intestinal lesions  
17 were present but less severe. However, ulcerative lesions among cattle surviving the clinical  
18 episode were shown to rapidly dissipate.<sup>1</sup>

19 A subsequent report indicated that a virus had been isolated from the previously  
20 mentioned cases and that the syndrome had been coined “viral diarrhea virus”.<sup>14</sup> These early  
21 observations and experimental findings were soon followed by additional reports describing  
22 similar clinical signs, outcomes, and evidence of transmissibility among herds in the U.S.,  
23 Canada, and Europe.<sup>10,12-19</sup>

24 In summary, the cumulative body of research provided a large degree of evidence that a  
25 novel, highly infectious and economically destructive disease process was prevalent in the  
26 bovine population. In addition, this clinical syndrome soon appeared to possess a worldwide  
27 prevalence.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31

## The Role of BVDV in Bovine Reproduction

Mature female beef cattle optimize their production by annually becoming pregnant, maintaining that pregnancy, and generating a live calf. Therefore, infectious and noninfectious etiologies negatively influencing the efficiency of this reproductive process can reduce the economic viability of the production system. The potential negative impact of BVDV on reproduction may be one of its most harmful (and economically devastating) characteristics. Early observations among BVDV researchers consistently associated reproductive wastage with the disease.<sup>1,11,12,20-23</sup> However, these early reports of abortion outbreaks (supposedly linked with prior bouts of clinical and subclinical “viral diarrhea”) were merely circumspect as authors were unable to isolate BVDV from aborted fetuses.<sup>1,11,12,18-20,22</sup> The theory that BVDV could indeed infect the unborn fetus was later reinforced by observing BVDV antibodies in fetal bovine serum harvested in slaughterhouses thereby suggesting that *in utero* exposure to BVDV had occurred.<sup>24</sup>

Further association of BVDV with reproductive loss was observed upon isolation of a non-cytopathic (NCP) BVDV from two aborted fetuses.<sup>25</sup> This finding was experimentally evaluated by Gratzek (1968) in which 12 fetuses (of different ages) were directly injected with BVDV while *in utero*.<sup>26</sup> Abortions were observed in six of the pregnant cows while the remaining fetuses were shown to possess pre-colostral BVDV antibodies at birth.<sup>26</sup> These findings were further applied in studies where the pregnant dam (not the fetus) was directly infected in order to externally validate the effects of BVDV on the unborn fetus. These studies utilized mature cattle that were in the last trimester of pregnancy. Although abortion failed to be observed, multiple calves displayed pre-colostral antibody titers to the same BVDV that infected the pregnant dams.<sup>21,27</sup>

These observations and experimental findings suggested that numerous outcomes were possible upon BVDV fetal infection and may be attributed to the timeframe of gestation of when infection occurred. Kendrick et al (1971) attempted to describe this variation by experimentally infecting pregnant cattle in all three trimesters of pregnancy.<sup>22</sup> Among the BVDV antibody negative cows infected while in the first trimester of pregnancy (n=9), three calves aborted, one was mummified, one was still-born, one was full-term with partial alopecia, and three appeared normal. Conversely, all cows in the second and third trimesters (n=14) delivered live healthy calves without evidence of abnormalities except for the presence of pre-colostral antibodies to



1 the virus. Although the authors were unable to isolate BVDV from any of aborted, stillborn, or  
2 normal calves (a finding that still frustrates diagnosticians today), these findings suggested that  
3 BVDV could indeed cause reproductive failure but that the outcome depended on when the dam  
4 was infected during her pregnancy. Dams infected early in pregnancy appeared to be at greater  
5 risk of pregnancy wastage compared to cattle in later stages of gestation.<sup>22</sup> These findings and  
6 hypotheses were soon confirmed by Casaro et al 1971 who infected cows with BVDV at various  
7 stages of pregnancy (51 – 256 days). These authors isolated BVDV in 23 of 26 fetuses and  
8 further displayed a trend of reduced clinical effects in older fetuses (second and third trimester)  
9 when compared to fetuses infected in the first trimester.<sup>23</sup>

10        Ensuing studies described the pathogenesis of pregnancy wastage attributed to BVDV.  
11 Studies evaluating the outcome of BVDV on the female reproductive tract, infertility, and early  
12 embryonic death suggest that the effects of BVDV during the first trimester of pregnancy are  
13 vast and may be attributed to direct oocyte damage, oophoritis (encompassing a reduction in  
14 follicular development and hormone production), or salpingitis/metritis which may significantly  
15 impact the dam's ability to maintain the pregnancy.<sup>28-36</sup> Other research also found that BVDV  
16 could induce abortion at any point of gestation.<sup>37-39</sup>

17        Alternatively, in addition to infertility and pregnancy loss, BVDV has also been  
18 associated with numerous congenital defects when *in utero* infection occurs during the timeframe  
19 of 100 – 150 days of gestation. This timeframe of infection appears to increase the probability of  
20 defects due to concurrent fetal organogenesis. Most reported defects predominately involve the  
21 central nervous system; specifically cerebellar hypoplasia,<sup>27,40-43</sup> microencephalopathy,<sup>44</sup>  
22 hydrocephalus,<sup>44</sup> hydranencephaly,<sup>44</sup> porencephaly,<sup>45</sup> and hypomyelination.<sup>46</sup> Ocular defects  
23 have also been commonly reported including cataracts,<sup>47,48</sup> microphthalmia,<sup>41,42,49</sup> retinal  
24 degeneration,<sup>41</sup> and optic neuritis.<sup>47</sup> Other ancillary reported defects include thymic  
25 hypoplasia,<sup>39</sup> hypotrichosis and alopecia,<sup>22</sup> mandibular brachygnathism,<sup>50</sup> and growth  
26 retardation.<sup>39,51</sup> Calves stricken with these abnormalities are severely handicapped at birth and  
27 frequently die soon thereafter or are subsequently euthanized.<sup>52</sup>

28        In summary, the effects of BVDV on pregnancy have been shown to be driven by the  
29 timing of infection with regard to fetal age. Previous research has observed that the bovine fetus  
30 and the status of the pregnancy are most susceptible to damage early in gestation. The risk of  
31 negative clinical implications is reduced in the latter stages of pregnancy.

## **The Manifestation and Clinical Implications of Cattle Persistently Infected with BVDV**

In addition to variable reproductive outcomes attributed to BVDV, fetal infection occurring early in gestation may lead to offspring that are immunotolerant and persistently infected (PI) with the virus. Persistent infections are attributed to fetal infection occurring prior to the development of a competent immune system by the fetus. Therefore, upon maturation of the fetal immune system, the preexisting BVDV infection is observed to be a “self” antigen and therefore the immune system fails to clear the viral infection. If the fetus survives gestation and is born alive, the calf is then a lifelong shedder of the virus thereby representing a highly infectious source of BVDV to herd and pen mates. Due to this consistent source of virus, PI cattle serve as the reservoir of BVDV infection.

The phenomenon of BVDV PI was first theorized by Kendrick (1970) who recommended that the concept of immunotolerance should be studied due to prior observations of cattle with mucosal disease (MD) being unable to produce antibodies to the BVDV of which it was infected.<sup>22</sup> Ensuing research recognized PI cattle; however, the significance of this class of cattle (i.e. lifelong viral distributors) was not fully appreciated as research was relegated to the immunological and clinical outcomes of individual PI animals.<sup>53,54</sup> The characterization of PI cattle as chronic shedders of BVDV was first observed in 1978 in a PI Holstein-Friesian bull.<sup>55</sup> The authors discovered the bull’s PI status at birth by the presence of BVDV in precolostral blood and synovial fluid samples. Intermittent sample collection from the bull throughout the ensuing two and a half years displayed BVDV in multiple bodily fluids including blood (buffy coat), nasal discharge, lacrimal secretions, and semen.<sup>55</sup> Immunocompetency for other pathogens was observed in the PI bull due to the production of antibodies to parainfluenza type 3 and bovine adenovirus despite failing to produce antibodies to BVDV throughout the study period.<sup>55</sup> The pathogenicity of the recovered BVDV from the PI bull was then demonstrated by exposing two colostrum deprived neonatal Holstein calves to the isolated virus. Both calves subsequently developed a fever and diarrhea lasting 5 – 7 days in duration and consequently produced antibodies one month after exposure to the BVDV of which they were inoculated.<sup>55</sup>

Although Coria and McClurkin (1978) theorized that the production of PI cattle was attributed to the timeframe of fetal infection,<sup>55</sup> McClurkin et al (1984) was the first to confirm this hypothesis.<sup>56</sup> Five experiments were performed in which different sample populations of

1 pregnant cows were exposed to distinct strains of BVDV (4 non-cytopathic [NCP] and 1  
2 cytopathic [CP]) at varied stages of pregnancy. Across the five experiments, BVDV was either  
3 injected directly into the amniotic fluid (via laparotomy) of the pregnant uterus or intravenously  
4 into the pregnant dam. The range of gestation among the study subjects was 42 – 125 days. The  
5 authors observed that the majority of fetuses exposed to NCP BVDV whom survived gestation  
6 were classified as PI to BVDV due to isolation of virus from the calves at parturition. Fetuses  
7 born to dams infected with CP BVDV showed no evidence of PI but displayed precolostral  
8 BVDV antibodies to the CP virus. All PI calves displayed immunocompetance to other antigens  
9 (bovine herpes virus, parainfluenza virus, and pasteurilla multocida) by developing antibodies to  
10 all except the BVDV of which they were PI.<sup>56</sup> The large majority of PI calves displayed normal  
11 disposition and subsequent growth. One PI heifer lived to breeding age and was observed to  
12 give birth to a PI calf; however, the majority of PI calves died prior to the onset of puberty.  
13 These data suggested that BVDV infection of the fetus between days 42 – 125 of gestation can  
14 result in the production of PI calves. Additionally, this study first suggested that PI cattle are at a  
15 high risk of dying prematurely; however, if females live until breeding age they may have a  
16 greater propensity to produce a PI calf.<sup>56</sup>

17 Early classification of BVDV outbreaks distinctly discerned “viral diarrhea” and  
18 “mucosal disease” (MD) as two separate entities based upon clinical outcome, pathological  
19 description (antemortem and post-mortem), and histopathological differences in ulcerations of  
20 the alimentary tract.<sup>10,11,15-17,19,57</sup> This theory was challenged by Thomson and Savan (1963) who  
21 experimentally induced “viral diarrhea” among neonatal calves resulting in clinical disease and  
22 virus isolation findings (i.e. cytopathic effects) typical of MD.<sup>58</sup> Based on their findings, the  
23 authors suggested that the two syndromes were likely associated with the same pathogen and  
24 both clinical syndromes should be universally classified as “viral diarrhea”. Further studies on  
25 PI cattle observed that only cattle displaying PI were shown to succumb to MD.<sup>54,59,60</sup> Brownlie  
26 et al 1984 provided the first investigational report detailing a possible etiopathogenesis of MD by  
27 observing that both NCP and CP BVDV could be isolated from PI cattle dying of mucosal  
28 disease.<sup>61</sup> Conversely, only NCP BVDV could be isolated from clinically normal PI cattle.  
29 Mucosal disease was then induced in two clinically normal PI calves by intra-nasal inoculation  
30 of a CP strain of BVDV. Both PI calves displayed severe anorexia and diarrhea within three  
31 weeks of exposure. Both calves were euthanized and post-mortem exams revealed lesions

1 consistent with mucosal disease. The authors hypothesized that mucosal disease requires the  
2 animal to be PI (with NCP BVDV) and subsequently “superinfected” with a CP BVDV.<sup>61</sup> This  
3 hypothesis was later confirmed by Bolin et al (1985).<sup>62</sup>

4 The transmission of BVDV is optimized when susceptible cattle have direct contact with  
5 the virus.<sup>63</sup> Potential sources of viral exposure include PI cattle,<sup>56,64-67</sup> transiently infected (TI)  
6 cattle,<sup>36,68-72</sup> wildlife,<sup>73-76</sup> and various fomites.<sup>77-80</sup> However, despite the various means of  
7 contracting BVDV, current beliefs indicate that exposure to PI cattle maximizes the risk of  
8 introducing or maintaining BVDV in the herd due to the concentration and consistency of viral  
9 shedding. Cattle PI with BVDV shed the virus in all bodily secretions and excretions for the life  
10 of the animal.<sup>56,63-65</sup> These data suggest that PI cattle are at risk of providing continual viral  
11 exposure to non-PI cattle thereby elevating the risk of morbidity, mortality, pregnancy wastage,  
12 and performance loss.

13 Despite the negative effects due to exposure to PI cattle, the prevalence of this  
14 subpopulation has consistently been shown to be low in both young stock and mature  
15 cattle,<sup>4,5,65,81-87</sup> although clustering of PI cattle can be observed at the reproductive herd  
16 level.<sup>84,88</sup> Persistently infected cattle have been observed to be at greater risk of overall  
17 morbidity/mortality when compared to non-PI cattle.<sup>89-91</sup> In addition, poor growth rates have  
18 been previously documented among PI cattle.<sup>2,92</sup> However, anecdotal reports suggest that many  
19 PI animals may appear clinically normally and indistinguishable from their non-PI counterparts.

20 Variation in the economic impact of BVDV tends to parallel clinical outcomes.  
21 Numerous case reports and cohort studies describe the negative clinical effects and performance  
22 loss attributed to PI cattle in both cow-calf and feedlot production systems.<sup>2,4,8,9,93-99</sup> Despite the  
23 reality that these clinical outcomes likely manifest in economic loss to the respective production  
24 system, there are limited peer-reviewed studies estimating the cost of BVDV in the cow-calf and  
25 feedlot sectors of the beef industry. Waldner et al 2008 observed that cow-calf herds with  
26 evidence of BVDV exposure among the calves (based upon antibody titers) displayed  
27 significantly reduced weaning weights (-21.6 lbs; 95% CI, -7.7 to -35.4) compared to herds  
28 without evidence of exposure.<sup>8</sup> Additionally, the risk of mortality was significantly greater in  
29 exposed herds compared to herds without evidence of BVDV; however, this effect was lost upon  
30 adjusting for the mortality of PI cattle.<sup>8</sup> In the feedlot, Hessman et al 2009 observed that the cost  
31 of exposure to a PI calf among non-PI cattle housed in the same or adjacent pen ranged from

1 \$41.84 – \$93.52 per head; this finding was driven predominately by performance losses  
2 (\$88.26/hd) and fatality (\$5.26/hd).<sup>9</sup>

3 Despite the quantity of literature depicting negative clinical and economic effects  
4 attributed to BVDV, several authors have failed to observe health or performance loss among  
5 cattle exposed to PI calves. O'Connor et al 2005 followed 5,041 calves (housed in 50 pens) from  
6 arrival to slaughter at a commercial Iowa feedlot.<sup>5</sup> The authors observed that the risk of  
7 morbidity among pens containing PI cattle and pens not containing PI cattle were not statistically  
8 different ( $P>0.05$ ).<sup>5</sup> Similarly, other researchers observed no statistical differences in the risk of  
9 morbidity and mortality as well as performance differences among commercial feedlot pens with  
10 or without PI cattle.<sup>6,7</sup> Elam et al 2008 observed no adverse effects (health, performance, and  
11 carcass quality) among pens of heifer calves exposed to PI cattle for both a short duration (60  
12 hours) or for the duration of the feeding period when compared to cattle not exposed to PI  
13 calves.<sup>100</sup> Additionally, the presence of PI cattle either in the pen or in adjacent pens did not  
14 appear to affect feedlot or carcass performance.<sup>100</sup>

15 The discovery of the ability of BVDV to persistently infect unborn fetuses was a major  
16 advancement in the realm of BVDV research by providing researchers and practicing  
17 veterinarians with a possible description of the viral reservoir. However, ensuing observational  
18 studies revealed a vast degree of variation in the clinical and economical outcomes among cattle  
19 exposed to PI individuals. Nonetheless, future control and programs would center on the  
20 identification and prevention of PI cattle.

## 21 **Genetic Variability of BVDV**

22 The initial 45 years of BVDV research focused on the pathogenesis of the virus, the  
23 discovery and clinical implications of PI cattle, and the institution of basic control programs  
24 based upon one viral genotype. However, in the early 1990's, new observations surfaced  
25 regarding a severe form of BVDV shown to manifest in a variety of novel clinical signs induced  
26 by thrombocytopenia including bloody diarrhea, epistaxis, petechial and ecchymotic  
27 hemorrhages, mucosal ulcerations, and injection site hemorrhages coupled with mortality risks as  
28 high as 10% – 20%.<sup>101-104</sup> Further research indicated that these severe outbreaks were  
29 subsequently associated with a genetic variant subsequently categorized as BVDV type II.<sup>105,106</sup>

1           Subsequent studies have revealed that not only does genetic variation delineate genotypes  
2 (types I and II) but variation within genotypes (i.e. subtypes) have also been identified.<sup>107-109</sup> In  
3 the U.S., prevalence studies have since observed that type I BVDV (specifically type Ib) is more  
4 prevalent in U.S feedlots than type II BVDV.<sup>108,110,111</sup> However, the realization of genetic  
5 variation of BVDV complicated and refuted previous notions that the efficacy of vaccine  
6 programs were sufficient and that mucosal disease could be diagnosed based solely on clinical  
7 signs (as mucosal ulcers were displayed on non-PI cattle with type II BVDV).<sup>112</sup> Like type I,  
8 type II BVDV was also observed to induce reproductive losses and produce PI cattle as a result  
9 of fetal infection.<sup>113</sup> However, the clear distinction between type II and type I infection was type  
10 II BVDV's ability to occasionally induce severe clinical disease and kill acutely infected cattle  
11 similar to the effect of type I in early reports. Further research suggested that type II clinical  
12 infections displayed a higher degree of virulence and clinical illness of longer duration compared  
13 to current type I BVDV.<sup>114</sup>

14           In summary, the discovery of genetic variation (both within and between genotypes) of  
15 BVDV appeared to explain some variation in clinical observations among cattle transiently  
16 infected with BVDV. However, this added variation complicated previous control programs that  
17 focused on vaccinating solely against type I BVDV. Future vaccine studies would focus on  
18 genotype cross protection and the need for type II BVDV (and multiple type I subtypes) to be  
19 included in commercial vaccine products.

## 20           **The Role of BVDV in the Bovine Respiratory Disease Complex**

21           The bovine respiratory disease (BRD) complex is the most prevalent disease in the  
22 feedlot sector of the U.S. beef industry with economic effects including costs of prevention,  
23 treatment costs, loss of feed performance and carcass quality, culling, and mortality.<sup>115-119</sup>  
24 Numerous pathogens have been shown to induce and increase severity of clinical BRD. In the  
25 feedlot setting, diagnostic investigations have repeatedly isolated BVDV from lung tissue of  
26 cattle with ante-mortem history (clinical signs and antimicrobial treatment) and post-mortem  
27 pneumonic lesions consistent with BRD.<sup>120-122</sup> However, the role of BVDV in the disease  
28 process has long been controversial and the mode of pathogenesis has not been entirely  
29 understood.<sup>123</sup> Nonetheless, the majority of current evidence indicates that the virus can play a  
30 significant role in the disease process.

1 As described above, the direct effects of BVDV can be extensive and have been observed  
2 to affect all age groups and classes of cattle. However, in addition to these disease syndromes,  
3 BVDV also possesses the ability to immunocompromise its host by a variety of methods.<sup>124-126</sup>  
4 The vital feature of immunosuppression (due to BVDV infection) is the magnitude of disease  
5 risk secondary to elevating the host's susceptibility to opportunistic pathogens. Indeed these  
6 immunosuppressive properties of the virus have been associated with concurrent disease  
7 processes.<sup>99,127-131</sup>

8 Cattle experimentally infected with BVDV (via endoscopic inoculation into the lungs)  
9 showed clinical symptoms (albeit mild when compared to cattle infected with both BVDV and  
10 *Mannheimia hemolytica*) thereby indicating that BVDV can directly cause BRD.<sup>132,133</sup> However,  
11 despite these findings, the majority of data suggests that the immunosuppressive characteristics  
12 of the virus (described above) which may increase the severity of clinical signs by potentiating  
13 infection with opportunistic pathogens is the main mode of action for BVDV associated  
14 BRD.<sup>129,131-134</sup>

15 In summary, veterinarians have long observed a wide array of clinical syndromes  
16 associated with BVDV. The earliest accounts of the disease describe a high prevalence of  
17 diarrhea and overall illness as well as reproductive consequences among adult  
18 animals.<sup>1,10,14,18,21,58,135</sup> Further studies added to the multi-dimensional nature of the virus by  
19 identifying different biotypes (NCP and CP),<sup>25,136</sup> the importance of those biotypes with regard to  
20 PI cattle production and mucosal disease,<sup>56,61,62</sup> recognizing its ability to genetically mutate while  
21 displaying changes in virulence,<sup>104,106,137</sup> observing that most infections are mild or  
22 subclinical,<sup>1,91</sup> the ability of the virus to compromise the immune system of its host thereby  
23 increasing the risk of concurrent disease,<sup>99,125,126,128,129,132,138</sup> and, finally, identifying its  
24 association with BRD.<sup>132,134</sup> Indeed, the name "viral diarrhea virus" is not only redundant but is  
25 also a misnomer based on its numerous clinical manifestations as well as the modern rarity of  
26 recognizing diarrhea being directly associated with BVDV infection. Despite these realizations,  
27 the variation in clinical outcome among different bovine populations is likely attributed to the  
28 potential genetic differences among BVDV strains, pre-existing immunity to BVDV, behavioral  
29 characteristics (i.e. the rate of contact) among populations housing infected cattle, and the level  
30 of viral exposure introduced to the population.<sup>104,139,140</sup>





1 should begin by screening the calf population. This should be followed by testing all herd bulls  
2 and open cows and ending with tests performed on the dams of test positive calves.<sup>143,152</sup> In the  
3 cow-calf sector, previous studies have indicated that the herd BVDV prevalence among U.S.  
4 beef herds is less than 10%.<sup>3,8,154</sup> However, the within herd prevalence of PI cattle among  
5 positive herds has been observed to be highly variable indicating that PI cattle tend to be  
6 clustered among cow-calf herds.<sup>2,3,8,154</sup> Due to the low herd prevalence, the majority of U.S.  
7 cow-calf herds may not economically benefit from implementing a whole-herd testing regimen.  
8 Previous data indicate that veterinarians are not highly accurate in diagnosing herd BVDV status  
9 based solely on clinical history.<sup>3</sup> Therefore, veterinarians should cautiously implement whole-  
10 herd testing protocols unless previous diagnostics have indicated that BVDV is circulating in the  
11 herd. Alternatively, negative herds may benefit from testing incoming replacement cattle and  
12 stocker cattle in order to reduce the risk of introducing BVDV to the herd.<sup>155-158</sup>

13 Despite the propensity of BVDV to inflict economic loss, veterinarians must be cognizant  
14 of the cost to the producer when instituting PI testing programs. Larson et al 2002 performed a  
15 10 year cow-calf profitability model that compared three herd BVDV scenarios: 1) beef herds  
16 with no PI calves, 2) herds with at least one PI calf displaying a negative impact on herd  
17 reproduction, and 3) herds with at least one PI calf displaying a negative impact on herd  
18 reproduction, pre-weaning morbidity, and pre-weaning mortality.<sup>141</sup> Their results indicated that  
19 herd BVDV prevalence highly dictated the potential profitability of instituting a BVDV testing  
20 program. These findings suggest that economic justification may not be present for the  
21 implementation of BVDV testing protocols in all herds.<sup>141</sup> Alternatively, veterinarians should  
22 evaluate the individual clinical histories of respective farms in order to gauge the likelihood of  
23 the testing protocol to produce a positive economic return.<sup>3,141</sup>

24 ***BVDV Vaccination***

25 A sound BVDV control program at the cow-calf level should include the administration  
26 of proven vaccine products in order to reduce the risk of fetal infection and clinical disease.  
27 However, realistic expectations regarding the vaccine’s ability to prevent BVDV infection  
28 should be thoroughly discussed between the veterinarian and the producer; unfortunately, failure  
29 to understand the limitations of a vaccine program may lead to frustration among beef  
30 producers.<sup>142</sup>

1           Despite different attributes among BVDV vaccines (i.e. modified-live and killed  
2 vaccines), it is imperative that labeled directions be followed. Veterinarians must be cognizant  
3 of the production system and thereby judiciously implement vaccines based on current  
4 management practices and vaccine history. Strategically administering vaccines optimizes the  
5 potential of disease prevention. In the cow herd, vaccines should be administered prior to  
6 breeding in order to reduce the risk of fertility complications, early embryonic death, fetal  
7 infection, pregnancy wastage (abortions, stillbirths), and congenital malformation.<sup>159-163</sup> Beef  
8 calves destined for feedlot systems should ideally be vaccinated well in advance of entry (e.g.  
9 prior to weaning) due to the stress and potential disease associated with that age bracket of  
10 cattle.<sup>164-167</sup>

11           In addition to knowledge of individual production systems, veterinarians must keep in  
12 mind the genetic variability inherent within the virus. The BVDV vaccine of choice should  
13 provide cross-protection against multiple BVDV isolates. Although prior studies have displayed  
14 that type I BVDV vaccines provide sufficient cross-protection against multiple type I subtypes as  
15 well as a type II challenge,<sup>168-171</sup> type II BVDV has been observed to induce disease and fetal  
16 infection with subsequent PI calf production among cow-calf herds administered a BVDV  
17 vaccine containing only the type I genotype.<sup>172</sup> Presently, multiple commercial vaccines (both  
18 MLV and killed) contain both BVDV genotypes and have displayed efficacy in preventing fetal  
19 infection and clinical disease.<sup>163,173,174</sup> The high prevalence of BVDV type 1b in U.S. feedlots  
20 has highlighted the possible need of incorporating that subtype into commercial vaccine products  
21 (the majority of which include only type 1a as the representative of the type 1 genotype).<sup>66,108</sup>  
22 However, recent data illustrates the potential of commercial vaccines (without BVDV type 1b) to  
23 prevent fetal infection among previously vaccinated heifers exposed to BVDV type 1b.<sup>175</sup>

24           In the cow-calf sector, veterinarians implement vaccine protocols to not only reduce  
25 clinical disease but to safeguard against fetal infection. Presently, multiple commercial vaccines  
26 (both MLV and killed) contain both BVDV genotypes and have demonstrated efficacy in clinical  
27 trials by preventing fetal infection and clinical disease.<sup>163,173,174</sup> However, veterinarians and  
28 producers should be wary of relying solely on vaccines in the prevention of fetal infection as  
29 multiple studies have shown that vaccines fail to provide 100% protection against disease  
30 challenge.<sup>113,159,161,163,175,176</sup> Nonetheless, veterinarians should base their vaccine choice on

1 published literature, maximizing the genetic variation within the vaccine, prior experience, and  
2 the practicalities of each individual production system.

### 3 *Biosecurity*

4 A sound biosecurity program is likely the most effective measure at either maintaining a  
5 negative BVDV status and/or reducing the risk of viral exposure to the resident herd.<sup>142,157,158</sup>  
6 Unfortunately, this component of BVDV prevention can be difficult to implement and maintain  
7 due to lack of producer awareness regarding the risk of disease introduction associated with  
8 current production practices, the lack of facilities to institute a biosecurity program, and a lack of  
9 appreciation for its importance. Nonetheless, veterinarians must stress the significance of this  
10 component in order to supplement diagnostic testing and vaccination protocols to further reduce  
11 of the risk of introducing BVDV to the herd. The implementation of a quality biosecurity  
12 program is typically accomplished by first identifying potential avenues for disease introduction,  
13 evaluating the necessity of those practices and potential alternatives, and managing them  
14 accordingly.<sup>142</sup>

15 The introduction of BVDV into a previously negative cow herd is commonly attributed to  
16 the introduction of new cattle.<sup>142,157,158</sup> Ideally, herd additions should be quarantined from the  
17 resident herd, screened for BVDV upon arrival, and maintained in an isolated location until test  
18 results are received and transient infections have likely cleared.<sup>142,155,157</sup> The risk of importing an  
19 adult PI animal has shown to be very low;<sup>85-87</sup> however, cattle acutely infected with BVDV can  
20 shed the virus making them potentially infectious to members of the resident herd. The  
21 introduction of stocker cattle poses an additional risk due to an increase in the prevalence of PI  
22 cattle among stockers and the sheer number of individuals entering the herd. Importation of  
23 pregnant cattle may increase the risk of BVDV introduction due to the unknown PI status of the  
24 unborn fetus. Smith et al 2008 displayed that importation of either or both of these  
25 aforementioned classes of cattle greatly increased the likelihood of introducing BVDV to the  
26 resident herd.<sup>155</sup> However, the risk associated with stocker cattle was reduced by testing all  
27 stocker calves for BVDV upon arrival and testing the offspring of imported pregnant cattle at  
28 parturition.<sup>155</sup> Preventing fenceline contact and/or communal grazing between the stockers and  
29 the resident herd may sufficiently offset this risk without incurring the test cost. Conversely, to  
30 mitigate the risk of the unborn fetus, incoming pregnant females should not only be quarantined

1 and confirmed BVDV negative but should be isolated from the resident herd until BVDV status  
2 of the calf is confirmed upon parturition.<sup>142,155</sup>

3 In addition to the management practices described above, additional practices that  
4 potentially increase the risk of BVDV introduction include fenceline contact with neighboring  
5 herds,<sup>157,158</sup> communal pasture sharing among herds,<sup>157,158</sup> reintroduction of cattle to the resident  
6 herd after off-site activities (e.g. exhibition, breeding),<sup>142,157</sup> procurement of bull semen from  
7 private distributors,<sup>71,177-179</sup> and potential wildlife contact.<sup>73-76</sup> Most of these activities (with the  
8 exception of wildlife contact) can either be strictly avoided (e.g. buying semen only from  
9 commercial vendors) or can be handled by implementing biosecurity protocols to these classes of  
10 cattle that parallel those described above for replacement animals.

### 11 ***BVDV Risk Analysis***

12 The goal of adding risk analysis to the BVDV control program is to assist in the  
13 identification of the most cost-effective preventative program.<sup>156,180</sup> In other words, risk analysis  
14 is implemented to identify potential disease risks, determine the probability of their occurrence,  
15 determine the cost of those respective disease risks if they were to occur, estimate the cost of  
16 disease prevention, and approximate the magnitude of risk mitigation attributed to the prevention  
17 program.<sup>156</sup> Biologic processes inherently possess certain degrees of variation. One form of risk  
18 analysis, stochastic modeling, allows the user to incorporate the variation thus providing a more  
19 realistic outcome (i.e. distribution) among production systems (when compared to deterministic  
20 models) thereby generating the information needed to make sound decisions.<sup>156,181</sup>

21 In summary, optimal BVDV control programs consist of combining both the magnitude  
22 of risk reduction afforded by the disease control program with the cost of implementation.<sup>142</sup>  
23 Therefore, our goal as veterinarians is to ensure that BVDV control programs reduce the risk of  
24 an outbreak (or the magnitude if one should occur) but to also ensure that the program can be  
25 economically validated. Therefore, optimal BVDV control programs consist of not only risk  
26 management tactics (e.g. diagnostic tests, vaccine protocols, and biosecurity programs) designed  
27 to reduce the probability of introducing BVDV to the resident cow herd but also institute the  
28 practice of risk analysis which provides the tools to help quantify the potential impact of a  
29 certain risk.<sup>181</sup>

### 30 **Conclusion**

1           The history of BVDV is fraught with evidence of disease and production loss.  
2   Historically, BVDV has been associated with numerous clinical syndromes and possesses the  
3   ability to immunocompromise the host thereby leading to concurrent disease. Fetal infection can  
4   cause of wide array of outcomes including pregnancy wastage and congenital malformation.  
5   Additionally, fetal infection occurring between days 42 – 125 of gestation has been shown to  
6   produce immunocompetant calves that are subsequently PI for life and thereby shed variable  
7   concentrations of BVDV into their environment. Although the prevalence is low, PI cattle are  
8   thought to be the viral reservoir and must be identified and removed for optimal BVDV  
9   prevention. A high degree of variability among clinical and performance outcomes has been  
10   displayed among cattle exposed to PI animals. Therefore, a single recommendation for PI testing  
11   protocols, vaccine administration, and biosecurity practices may not always be in the best  
12   economic interest of the respective production system. In the cow-calf sector, BVDV control  
13   and prevention is practiced at numerous levels including the identification of PI cattle present in  
14   the resident herd (diagnostic testing), reducing the risk of fetal infection and abortion  
15   (vaccination), and prevention of introducing BVDV to the resident herd (biosecurity). Given the  
16   significant cost of these programs and the variability of disease magnitude, veterinarians have  
17   recognized that optimal BVDV control involves not only risk management practices but also risk  
18   analysis methods in order to identify the most cost-effective control programs. An optimal  
19   BVDV control and preventative program would account for both the risk of acquiring BVDV  
20   with the cost of control and prevention.

1

2                   **CHAPTER 2 - Variation in Viremic Concentration**  
3                   **among Cattle Persistently Infected with Bovine Viral**  
4                   **Diarrhea Virus**

5                   **Introduction**

6                   The potential of a non-cytopathic (NCP) bovine viral diarrhea virus (BVDV) to  
7 persistently infect (PI) the bovine fetus was first described by McClurkin et al (1984).<sup>56</sup> Since  
8 that time, much work has been undertaken to fully understand the clinical, epidemiologic, and  
9 economic impact of the PI animal.<sup>3,64,91,143,155,182-185</sup> Cattle PI with BVDV excrete the virus in all  
10 bodily secretions and excretions for the life of the animal.<sup>64,182-184</sup> This characteristic maximizes  
11 the efficiency of viral distribution to non-PI cattle thereby making PI cattle the main reservoir of  
12 BVDV.<sup>3,56,91</sup> Control programs focused on BVDV mitigation vary based upon current clinical  
13 scenario, PI prevalence, and BVDV risk avoidance preferences of the owner.<sup>143,155</sup> In order to  
14 significantly reduce the risk of BVDV infection, PI cattle must be identified and removed from  
15 non-PI cattle; therefore, multiple diagnostic tests have been developed and validated to identify  
16 PI cattle.<sup>65,145,146,148-151,186,187</sup>

17                   Despite the number of reports incriminating PI cattle and BVDV as a major threat to the  
18 health and productivity of the bovine industry,<sup>4,8,9</sup> multiple authors suggest that comprehensive  
19 PI testing is not a valid economical endeavor due to the low prevalence of PI cattle.<sup>141,155,188</sup> In  
20 addition, other reports suggest that PI cattle may not be consistently relevant in the feedlot when  
21 comparing pens of cattle housing a PI(s) to pens without PI cattle.<sup>5,7,100</sup> This discrepancy in the  
22 literature is perplexing, but may suggest that all PI cattle are not equally effective in transmitting  
23 BVDV. Brock et al (1998) descriptively reported that the viremic concentration of BVDV  
24 appeared to change over a two-year time-frame in seven PI yearling cattle.<sup>183</sup> Therefore, one  
25 hypothesis for varied health outcomes in cohorts of cattle following PI exposure may be  
26 variability in the amount of BVDV shed by PI cattle. However, to our knowledge, there are no  
27 published reports of changes in viremia levels of PI cattle over time as determined from multiple  
28 serum samples taken at regular intervals. The objectives of this study were two-fold. The first  
29 objective was to determine if serum viral concentrations of BVDV differed among multiple PI

1 cattle. The second objective was to determine if BVDV serum viral concentration of PI cattle  
2 differed significantly over time. Three studies were designed to answer these questions.

### 3 **Materials and Methods**

4 All live animal studies were conducted and samples collected using protocols approved  
5 by the Kansas State University (KSU) Institutional Animal Care and Use committee.

#### 6 *Study 1*

7 Study one was a cross-sectional study composed of 12 mixed breed beef calves averaging  
8 840 lbs (381 kg) and 21 mixed breed beef calves averaging 353 lbs (160 kg) housed at two  
9 different commercial feedlots within the state of Kansas. All calves were maintained within  
10 open-air, dirt floor pens. Upon entry to the respective feedlot, a presumptive diagnosis of BVDV  
11 PI was determined by antigen capture ELISA (ACE) of ear tissue. These BVDV PI calves were  
12 subsequently isolated from the general feedlot population and housed together. A second ACE  
13 was performed at least four weeks after initial testing to confirm PI status on the suspect cattle.  
14 On the day of confirmatory sample collection, one six milliliter (ml) whole blood sample was  
15 collected by jugular venipuncture in a sterile clot tube from each individual calf. Within 24  
16 hours of sample collection, all samples were centrifuged at 3700 rpm for 10 minutes. The serum  
17 was harvested into individual cryovials and stored at -176° F (-80° C) until virus isolation (VI)  
18 was performed.

#### 19 *Study 2*

20 Six mixed-breed beef calves averaging 450 lbs (204 kg), presumptively diagnosed as PI  
21 with BVDV, were identified and managed by typical industry standards in a commercial Kansas  
22 feedlot. These calves were initially diagnosed by one ACE test on ear biopsy specimens and  
23 subsequently removed from the general population and fed in an isolated pen for the duration of  
24 the feeding period. Inclusion criteria for Study 2 consisted of two factors: 1) a positive follow-  
25 up ACE performed at least four weeks after the first ACE test and 2) two positive virus isolation  
26 (VI) tests two to four weeks apart. Sample collection was performed every seven days for one  
27 month by the feedlot's consulting veterinarian. Whole blood was collected by jugular  
28 venipuncture in sterile clot tubes (five mls) and EDTA tubes (four mls). These specimens were  
29 mailed overnight to the Kansas State Veterinary Diagnostic Lab (KSVDL). Plasma and serum

1 were harvested immediately following receipt of samples and stored at -176° F (-80° C) until VI  
2 was performed.

### 3 *Study 3*

4 Four mixed-breed beef calves averaging 485 lbs (220 kg) were diagnosed as presumptive  
5 PI cattle by one ACE on skin biopsy. Confirmation of PI status following arrival to KSU for this  
6 study was achieved by the same inclusion criteria and diagnostic regimen as Study 2. Calves  
7 were individually housed in outdoor pens measuring 20 square feet at a KSU animal resource  
8 facility. A minimum of 12 feet (3.66 meters) was placed between each pen to avoid direct  
9 contact between calves and thereby limit potential cross-contamination between pens.<sup>185</sup> Each  
10 individual pen was equipped with a small holding chute to secure the respective calf as well as  
11 individual feed and water sources. Disposable sleeves, gloves, and boots were worn to avoid  
12 cross-contamination between PI calves and new apparel was used for each pen. Five mls of  
13 blood were collected in red-top clot tubes and four mls of blood were collected in tubes  
14 containing EDTA by jugular venipuncture from each calf approximately every three days for 45  
15 days. Serum and plasma were harvested immediately following sample collection and were  
16 stored at -176° F (-80° C) until VI was performed.

17 Calves in study 3 were observed twice daily by a veterinarian to monitor for clinical signs  
18 of disease. If symptoms warranted further investigation, animals were individually removed  
19 from their pens and walked to the processing facilities where a thorough physical examination  
20 was performed by the attending veterinarian. Treatment of bovine respiratory disease (BRD)  
21 consisted of tulathromycin<sup>a</sup>, enrofloxacin<sup>b</sup>, and oxytetracycline<sup>c</sup> for the first, second, and third  
22 treatments, respectively. Feed bunks were observed daily and the amount of feed distributed to  
23 each calf was allocated based on the consumption in the period since the last feeding. Total  
24 amount (as fed) of daily feed delivery to each pen was recorded. Water was available *ad libitum*  
25 throughout the trial.

### 26 *Virus Isolation*

27 Media for cell culture was produced by adding five mls of Penicillin-Streptomycin, two  
28 mls of Ciprofloxacin, five mls of Amphotericin B, 20 mls of L-glutamine and 70 mls of fetal  
29 bovine serum to one liter (L) of EMEM. This mixture was inverted several times and set aside.  
30 Embryonic bovine lung (EBL) cells were washed with 12 mls of PBS prior to the addition of



1 four mls of Trypsin. The flask was then incubated for 5-15 minutes. The EBL cells were then  
2 resuspended in the cell culture media composed above. The volume of media was determined by  
3 the number of plates needed (approximately 10mls/plate). One hundred micro-liters ( $\mu$ ls) of the  
4 media/EBL cell mixture were added to each well in 96 well micro-titer plates. The plates were  
5 then incubated for two to six hours. Six ten-fold serial dilutions in quadruplicate were performed  
6 for each sample in four ml plastic tubes. A volume of 1.8 mls of media/EBL cell mixture was  
7 added to each tube. Two hundred  $\mu$ ls of the first sample (serum) was added to the first tube and  
8 vortexed. Two hundred  $\mu$ ls from the first tube was then removed and added to the second tube  
9 and vortexed. This process continued until all six dilutions had been created. One hundred  $\mu$ ls  
10 of each respective sample/dilution were then added to the respective well in each plate. The  
11 plates were then incubated for 48 hours. The media in each plate was then discarded and the  
12 remaining cells were washed with FA-PBS. An 80% acetone solution was then poured into each  
13 well and was allowed to sit for 10 minutes. Upon discarding the acetone, the plates were  
14 allowed to dry at room temperature. Fifty  $\mu$ ls of anti-BVDV conjugate was added to each well  
15 and allowed to incubate for one hour. The conjugate was then discarded and the plate washed  
16 with FA-PBS. Fifty  $\mu$ ls of 50% glycerol were added to each well. The cell-culture infectious  
17 dose (CCID-50) was calculated by a previously described method.<sup>189</sup>

## 18 *Statistics*

19 The individual calf was the unit for all analyses. Descriptive statistics were generated  
20 using a commercial software package<sup>d</sup>. Confirmation of homogeneity among the variance of the  
21 fixed effects was performed by general linear models<sup>e</sup>. Differences in BVDV serum  
22 concentrations among groups (Study 1), individual calves (Studies 2 and 3), and over the time  
23 (Studies 2 and 3) were evaluated by general linear mixed models<sup>f</sup>. A first-order autocorrelation  
24 structure was used to account for the lack of independence among subsequent samples from the  
25 same animal (Studies 2 and 3). All CCID-50 values were transformed to  $\log_{10}$  outcomes to  
26 facilitate the normalization of data. An alpha level of 0.10 was used for all analyses.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

## **Results**

### ***Study 1***

All 33 calves from both locations were confirmed to be PI based on the aforementioned inclusion criteria. A statistical difference was not observed between serum BVDV concentrations (CCID-50) of cattle from the two locations. The distribution of serum BVDV concentrations ranged from  $10^{1.75}$  to  $10^{4.5}$  among calves (Figure 4.1).

### ***Study 2***

All six calves in this study were confirmed to be PI by the inclusion criteria. Serum BVDV concentrations across the sample population ranged from  $10^{1.75}$  to  $10^{3.75}$  throughout the time period of the study (Figure 4.2). In this study population, one calf (#6600) exhibited significantly lower serum BVDV concentrations throughout the study compared to the other five; however, no significant differences were detected among the other five calves. No differences were observed in serum BVDV concentration across weekly measures in this study.

### ***Study 3***

Of the four calves composing this study, one calf was found to be transiently infected with BVDV while the other three calves were confirmed to be PI based on the aforementioned inclusion criteria. One PI calf succumbed to mucosal disease, confirmed by the KSVDL, shortly after the initiation of the study; therefore, data from this calf were not included in the analysis. Among the sample population, the distribution of raw CCID-50 values over the 45 day collection period was  $10^2$  to  $10^4$  virus particles per ml of serum. Within individual calves, serum viral concentrations over time were observed to fluctuate from  $10^{1.75}$  to  $10^4$  during the study timeframe. Significant differences were detected in BVDV viremia levels between individual calves ( $P=0.03$ ) and over time ( $P=0.10$ ). Figure 4.3 depicts the raw BVDV serum concentration of both calves at each collection time point. The model-adjusted serum BVDV concentration estimates were observed to significantly differ at adjacent sample collection time points of days 1 and 3, 35 and 38, and 38 and 41.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

## Discussion

Our results suggest that the BVDV serum concentrations in PI cattle have a relatively large distribution (Study 1), differ among PI cattle (Studies 2 and 3), and may change over time (Study 3). Previous reports have descriptively illustrated that BVDV serum concentration ranges among PI cattle<sup>190</sup> and may change over time<sup>183</sup>; however, to our knowledge, the present study is the first to quantitatively assess both factors. The variability in BVDV serum concentration among PIs and over time identified in this study may help explain reported variability in population responses following exposure to PI cattle.<sup>4,5,7-9,100</sup>

The methods implemented in this study for determining BVDV serum concentration have been well documented.<sup>65,183,191-193</sup> Descriptively, the ranges in serum BVDV concentrations among PI cattle from our studies are similar to a previous study which reported a distribution of serum BVDV concentrations of  $10^2$  to  $10^4$  among 12 PI calves at one time point.<sup>65</sup> Conversely, other studies have reported smaller distributions in serum viral concentrations; however, they tend to overlap those reported in the present study. Bolin et al (1985) reported serum BVDV concentrations ranging from  $10^4 - 10^{5.8}$  virus particles per ml among nine two-year old mixed breed PI steers prior to receiving a modified-live vaccine.<sup>191</sup> Patel et al (2002) observed serum titers of BVDV among four neonatal PI calves ranging from  $10^{2.2} - 10^{2.5}$  soon after birth.<sup>68</sup> Although large ranges have been described, we are unaware of published data documenting statistical differences in BVDV serum concentration among PI individuals and over time.

In both studies 2 and 3, we were able to identify significant differences in BVDV serum concentration among individual PI calves. One cause of these differences could be the presence and concentration of maternal antibodies to the homologous strain of BVDV still circulating at the time of sample collection. Palfi et al (1993) reported a lack of detectable virus in 11 newborn PI calves until six to eight weeks of age when BVDV maternal antibodies were observed to dissipate.<sup>194</sup> Likewise, Brock et al (1998) observed a lack of serum BVDV in three of four neonatal PI calves until approximately 42 days of age when maternal BVDV antibodies waned.<sup>183</sup> The level of maternal antibodies may influence serum BVDV concentrations, and although the PI calves in the present study were likely much older than the animals in these two reports, varied antibody levels may have played a role in our findings.

1 Another possible source of variation in serum BVDV concentrations may be attributed to  
2 spontaneous production of antibodies within the PI individual to the homologous strain of  
3 BVDV. Brock et al (1998) described the first account of developing virus neutralizing  
4 antibodies to the homologous strain of BVDV, within a three year old PI steer, thereby  
5 preventing the isolation of BVDV in serum by VI over an 18 month period of time.<sup>183</sup>  
6 Additionally, Grooms et al (2001) observed transmission of BVDV between an eight-year-old PI  
7 cow housed with a non-PI steer in which BVDV could not be identified in the PI cow's serum  
8 (but was present in buffy coat and nasal swab specimens).<sup>82</sup> Despite these reports, PI cattle  
9 lacking detectable levels of serum BVDV due to homologous antibody production likely  
10 represents a small proportion of the PI population. Further research is needed to determine why  
11 variation exists among serum BVDV concentrations of PI cattle.

12 In addition to serum BVDV concentration disparities among individual PI cattle, our data  
13 also suggest that serum BVDV concentrations significantly fluctuate over time (Study 3). Our  
14 findings agree with previous work that described an average decline in serum BVDV  
15 concentration of one log<sub>10</sub> among five of seven adult PI animals, over a two year period.<sup>183</sup>  
16 Similar results were reported by Fulton et al (2009) describing a variation of one to two log<sub>10</sub>  
17 changes in serum BVDV concentration in several PI calves during an 11 month study.<sup>190</sup>

18 The cause of the observed differences in serum BVDV concentrations over time are  
19 currently unknown, but are likely associated with a number of factors. One potential explanation  
20 for fluctuation in serum virus concentrations may be various forms of stress, which have been  
21 shown to be detrimental in both animal and human viral infections. Stress attributed to  
22 hyperthermic conditions and transportation has been associated with the recrudescence of herpes  
23 viruses in both the human and bovine species.<sup>195,196</sup> Additionally, the stress of actively being  
24 restrained has been observed to increase the distribution of Theiler's disease in humans.<sup>197</sup> All  
25 cattle included in the present study were restrained by standard industry processing facilities in  
26 order for sample collection to proceed. Despite these data, the role of stress and environmental  
27 conditions in the variation of serum BVDV concentration among PI cattle is currently unknown.  
28 More work is needed to determine what factors may influence the variation in serum BVDV  
29 concentration over time among PI cattle.

30 Despite the differences in serum BVDV concentrations among PI calves, these findings  
31 may not necessarily predict the viral load that non-PI cattle may encounter when exposed to a PI

1 individual. Prior studies suggest that serum BVDV concentrations approximate viral levels in  
2 nasal secretions, urine, feces, and uterine secretions.<sup>64,65</sup> However, Fulton et al (2009) observed  
3 that BVDV concentrations in nasal swabs were greater ( $10^{2.55} - 10^{3.50}$  differences in some cases)  
4 than serum BVDV concentrations and BVDV could be isolated from individual nasal swabs  
5 even when respective serum BVDV levels were undetectable by VI.<sup>190</sup> Further research is  
6 necessary to determine if serum BVDV concentration is associated with the level of virus shed  
7 into the environment.

8

9

1

2

### **Sources and Manufacturers**

3

a. Draxxin<sup>®</sup>, Pfizer Animal Health, Kalamzoo, MI

4

b. Nuflor<sup>®</sup>, Intervet/Schering Plough Animal Health, DeSoto, KS

5

c. Biomycin 200<sup>®</sup>, Boehringer Ingelheim, Saint Joseph, MO

6

d. Microsoft Excel<sup>®</sup>, 2003, Redmond, WA

7

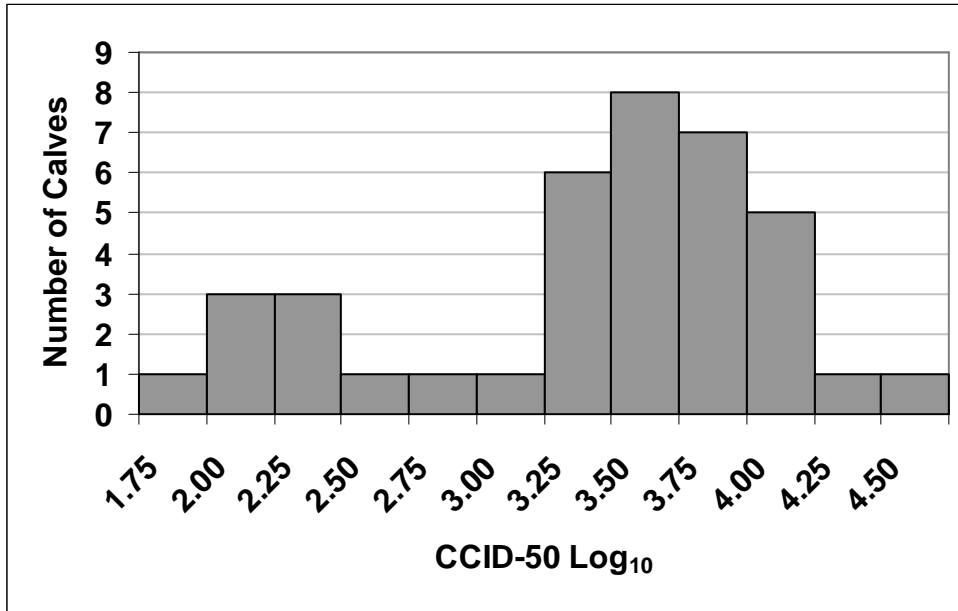
e. PROC GLM, SAS Institute, Cary, NC

8

f. PROC Mixed, SAS Institute, Cary, NC

1

2 **Figure 2.1: Distribution of BVDV serum concentrations at one point in time among 33 PI**  
3 **animals composing Study 1.**

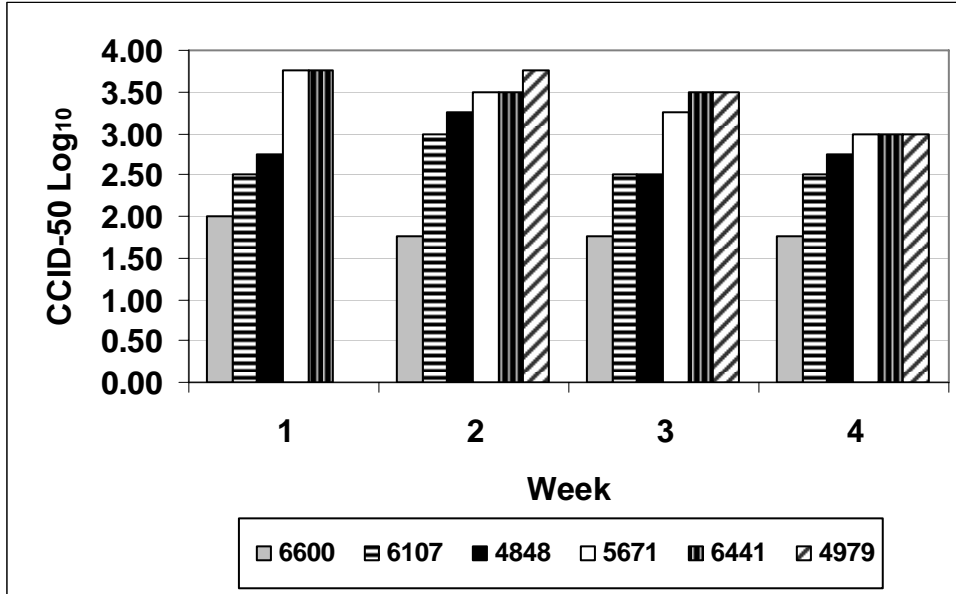


4

5

1 **Figure 2.2: Individual serum BVDV concentrations among PI calves (n=6) in Study 2**  
2 **measured weekly by VI for four weeks. Note that calf # 4979 is missing data in Week 1.**

3

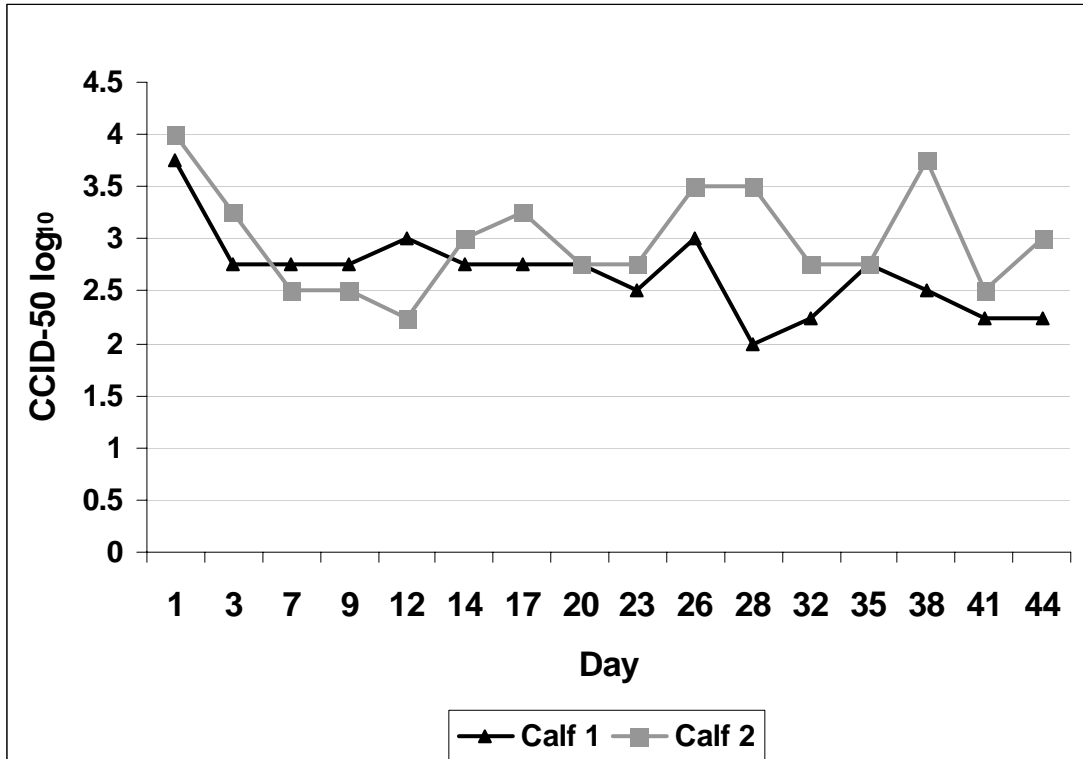


4



1 **Figure 2.3: Individual serum BVDV concentration for two PI calves at each sample**  
2 **collection time point across a 45 day study period.**

3



4

1                   **CHAPTER 3 - Evaluation of health and performance**  
2                   **outcomes in stocker calves following the administration**  
3                   **of a commercial vaccine containing a bovine viral**  
4                   **diarrhea virus contaminant**

5                   **Introduction**

6                   The strategic administration of commercial vaccines to cattle of all ages is a standard  
7                   practice of general preventative veterinary medicine in the United States. These products are  
8                   composed of killed or attenuated organisms intended to improve the health and production of  
9                   cattle.<sup>185,188,198</sup> Vaccine purity is a constant concern due to the potential contamination of  
10                  adventitious viruses, particularly virulent bovine viral diarrhea virus (BVDV).<sup>198,199</sup> In the  
11                  United States, BVDV is the most common contaminant of cell cultures used in the production of  
12                  viral vaccines.<sup>200</sup> The primary source of BVDV contamination is attributed to the utilization of  
13                  fetal bovine serum (FBS) in viral vaccine production.<sup>201</sup> Previous data reports the prevalence of  
14                  BVDV infected FBS to range from 10% to 87% of tested one-liter lots.<sup>202-206</sup>

15                  During the summer of 2008, Schering Plough Animal Health (SPA) confirmed that  
16                  specific serials of two commercial vaccine lines<sup>a,b</sup> were contaminated with an extraneous type II  
17                  NCP BVDV. Two Kansas State University (KSU) beef cattle animal health studies employed  
18                  one of the aforementioned vaccines<sup>a</sup> (confirmed contaminated serial numbers) without  
19                  knowledge of the BVDV contaminant. The Fall 2007 study was originally conducted to evaluate  
20                  metaphylactic protocols<sup>207</sup> and incidence of BVDV transient infections in 293 beef feeder calves.  
21                  The objective of the Spring 2008 trial was to observe 22 cattle post-vaccination to evaluate the  
22                  potential of PCR to identify the BVDV fraction of the vaccine. Both studies collected health,  
23                  performance data, and blood samples from all cattle at numerous time-points.

24                  A retrospective cohort study was performed on stock-piled serum samples and data to  
25                  analyze health and performance outcomes given the knowledge that the MLV vaccine contained  
26                  an extraneous BVDV. The objectives of this study were to evaluate the risk of contaminant  
27                  BVDV viremia after vaccination, the duration of contaminant BVDV viremia, the association of  
28                  pre-exposure BVDV serologic status and BVDV viremia, and potential associations of the  
29                  contaminant BVDV viremia with health and feed performance in beef calves.

## Materials and Methods

Samples from two independent research trials were used to conduct the retrospective analysis. The details of each trial are provided in addition to testing procedures conducted specifically for the current analyses. All animals in both trials were handled in accordance with protocols approved by the Kansas State University Animal Care and Use Committee.

### *Fall 2007 Trial*

Two-hundred ninety three mixed-breed beef bulls and steers of weaning age were procured in the southeastern United States and arrived to the KSU Beef Stocker Unit in three loads. The facility housing the cattle is an all-in-all-out system thereby reducing the risk of extraneous sources of microbial exposure. Upon arrival, cattle were individually weighed, identified (i.e. numbered ear tag), and gender (bull or steer) was determined. Cattle were blocked by weight and gonadal status and randomly assigned to one of eight pens for each truckload by a computer generated random number system<sup>c</sup>. The first pen in each string was assigned to metaphylaxis treatment A or B by a coin flip. Subsequent pens received alternating treatments.

Twenty four hours after arrival, each calf received a two milliliter (mL) commercial MLV 4-way viral respiratory vaccine<sup>a</sup>, a two mL 7-way clostridial vaccine<sup>d</sup>, injectable ivermectin<sup>e</sup>, and one of two metaphylaxis treatments (A: tilmicosin<sup>f</sup> at 10mg/kg by subcutaneous (SC) injection, B: tulathromycin<sup>g</sup> at 2.5mg/kg by SC injection) and bulls were surgically castrated as described in a previous report.<sup>207</sup> Two blood samples consisting of four mLs in EDTA tubes<sup>h</sup> and five mLs in clot tubes<sup>i</sup> were collected from each calf at arrival. In addition, a 1.5 squared centimeter (cm<sup>2</sup>) ear biopsy was collected from each calf by a standard ear notching device.<sup>j</sup> Calves were readministered the same MLV respiratory vaccine<sup>a</sup> and 7-way clostridial vaccine<sup>d</sup> 10 days after arrival. Blood was again collected from all calves at revaccination in the same fashion as at arrival. All products were administered per label directions. Twice daily, cattle were fed a total mixed ration consisting of prairie hay, alfalfa, wet gluten feed, and cracked corn. Feed bunks were observed once daily and the amount of feed distributed to each pen was based on the bunk score from that day. Automatic watering systems were present in each pen and water was available *ad libitum* throughout the trial.

1 Each pen was observed twice daily by trained personnel to identify individual animals  
2 displaying clinical signs of disease. A 72 hour post-metaphylactic treatment moratorium was  
3 observed for all cattle. The case definition for cattle experiencing bovine respiratory disease  
4 (BRD) included observance of clinical signs and a rectal temperature of  $\geq 40^{\circ}\text{C}$  ( $104^{\circ}\text{F}$ ). Cattle  
5 meeting the treatment criteria were treated with enrofloxacin<sup>k</sup> (7.5-12.5mg/kg, SC), florfenicol<sup>l</sup>  
6 (40mg/kg, SC), and oxytetracycline<sup>m</sup> (20mg/kg, SC) for the first, second, and third treatments,  
7 respectively. In addition, blood samples were collected from all calves treated for BRD. Calves  
8 designated with chronic respiratory disease (cattle meeting the BRD case definition after three  
9 treatments) were removed from their respective pen and placed in a pen housing other calves  
10 experiencing chronic BRD. Gross post-mortem examinations were performed on all dead calves  
11 at the Kansas State Veterinary Diagnostic Laboratory (KSVDL). At the conclusion of the  
12 feeding phase, cattle were weighed individually on the day they left the facility.

13 Within 1 hour following blood collection, plasma and serum were harvested from each  
14 respective blood sample. Blood (serum) and ear biopsy samples collected at arrival were tested  
15 for BVDV by real-time reverse-transcriptase polymerase chain reaction (PCR)<sup>n</sup> and antigen  
16 capture ELISA (ACE)<sup>o</sup>, respectively, on the day of arrival. Plasma and serum collected from  
17 samples obtained at revaccination and from each calf treated for BRD was stored at  $-80^{\circ}\text{C}$ . All  
18 samples were analyzed for BVDV by PCR at the end of the study period.

### 19 *Spring 2008 Trial*

20 Twenty two mixed breed beef steers of weaning age were procured at a local livestock  
21 market (within 60 miles) and transported to Manhattan, KS. All calves were housed in a single  
22 pen (2,090 m<sup>2</sup>) and isolated from other cattle throughout the duration of the study. Calves were  
23 randomly assigned to receive either a MLV 4-way respiratory vaccine<sup>a</sup> (n=16) or sterile saline<sup>p</sup>  
24 (n=6) based on results from a random number generator<sup>c</sup>.

25 Two hours after arrival, each calf received its predetermined treatment (MLV vaccine or  
26 sterile saline), a two mL multivalent clostridial vaccine<sup>q</sup>, and injectable doramectin<sup>r</sup>. All  
27 products were administered per label directions. In addition, blood and ear biopsy specimens  
28 from each calf were collected in the same manner as calves in the Fall 2007 study. Blood  
29 samples also were collected from all individuals in both treatment groups on days 7, 14, and 21  
30 post-vaccination. Within 1 hour following sample collection, serum and plasma were harvested

1 from each respective whole blood sample and stored at -80°C until the end of the study period.  
2 Serum samples and ear biopsies were subsequently tested for BVDV by PCR<sup>n</sup> and ACE<sup>o</sup>,  
3 respectively.

4 Each pen was observed twice daily by a veterinarian to identify sick cattle. Calves  
5 exhibiting clinical signs of BRD were further evaluated by a complete physical examination.  
6 Cattle registering rectal temperatures  $\geq 40^{\circ}$  C (104° F) were treated with tulathromycin<sup>g</sup> (2.5  
7 mg/kg SC), florfenicol<sup>l</sup> (40mg/kg, SC), and oxytetracycline<sup>m</sup> (20mg/kg, SC) for the first, second,  
8 and third treatments, respectively. At the conclusion of the study, all cattle left the facility on the  
9 same day.

### 10 *Serology*

11 Serum neutralization assays for type 1 and type 2 BVDV were performed on serum from  
12 both studies at the KSVDL by a previously described method.<sup>208</sup> Antibody titers were quantified  
13 on arrival serum samples from cattle in the Fall 2007 trial and on multiple samples (arrival, day  
14 7, day 14, day 21) from cattle in the Spring 2008 study. A titer  $\geq 1:4$  was classified antibody-  
15 positive to BVDV throughout the data analysis.<sup>66</sup> Four classifications of type 1 and type 2  
16 serology results were implemented: cattle without antibody titers to both BVDV genotypes,  
17 cattle positive for type 1 antibodies but not type 2, cattle positive for type 2 antibodies but not  
18 type 1, and cattle antibody-positive to both genotypes.

### 19 *Real-Time Polymerase Chain Reaction*

20 Individual serum samples were pooled (up to 10) to create a volume of 200 micro-liters  
21 ( $\mu$ l). Viral RNA was subsequently extracted using a commercial method<sup>s</sup> for PCR per the  
22 manufacturer's recommendations. Utilizing a commercial PCR kit<sup>n</sup>, five  $\mu$ l of the extracted viral  
23 RNA was added to 15  $\mu$ l of master mix to comprise a total volume of 20  $\mu$ l. The PCR was  
24 performed by a commercial system<sup>y</sup> as follows: reverse transcription at 45°C for 30 minutes,  
25 denaturation at 95° C for 2 minutes, 45 cycles of denaturation at 95°C for 30 seconds, and  
26 annealing/extension at 55°C for 60 seconds. The BVDV specific amplification is detected by  
27 FAM (6-carboxyfluorescein) fluorescence. To identify false negative results by inhibited PCR,  
28 the above mentioned kit contains an internal control in primer-probe-mix that is detected by VIC  
29 (2,7- dimethoxy-4,5-dichloro-6-carboxy-fluorescein) fluorescence. Only when the internal  
30 control was positive and the PCR was non-inhibitory were its results were included in this study.

1 Positive and negative controls from the kit were included in each PCR. At the end of each cycle,  
2 FAM fluorescence was measured and the cycle threshold (CT) was determined as the cycle  
3 number at which the increase in fluorescence is exponential. When any of the pools were tested  
4 positive by PCR, each blood sample in those pools was extracted individually for PCR as  
5 described above.

6 In the Spring study, three PCR tests were performed on every sample at each time point  
7 and results were interpreted in parallel; therefore, a sample was considered positive when at least  
8 one result out of three was positive. A negative classification required negative results on all  
9 three PCR tests.

### 10 *RNA Sequencing*

11 Serum samples determined to be BVDV positive by PCR<sup>n</sup> were then tested by a genetic  
12 sequence method to further confirm the presence of the virus. Individual serum samples were  
13 subjected to a PCR plus a melt curve analysis.<sup>u</sup> The primers used (forward primer BVD-2F: 5'-  
14 CATGCCCTTAGTAGGAC-3', reverse primer 384: 5'-CCATGTGCCATGTACAG-3') were  
15 modified from a previous report.<sup>209</sup> These primers amplify a 283 bp fragment from both type 1  
16 and type 2 BVDV in the 5' UTR region of the BVDV genome. Samples with a melt curve at  
17 approximately 84°C were diluted 10-fold, and 1 µl of such diluted amplicons were used as  
18 templates for a regular PCR re-amplification to enrich the amplicon concentration, and to remove  
19 the SYBR Green dye that may interfere with sequencing reactions. The resulting products were  
20 purified using a commercial purification kit<sup>v</sup> and outsourced<sup>w</sup> for sequencing from both ends.  
21 Each pair of sequences was assembled into a 283 bp contig and compared with known type 1 and  
22 type 2 BVDV sequences to confirm the respective genotypes.

### 23 *Statistical Analysis*

24 The individual calf was the experimental unit throughout the data analysis. General  
25 linear models were used to estimate the least square means of continuous outcomes for  
26 significant effects from the Fall 2007 study.<sup>x</sup> Repeated measures (on pen and lot) were  
27 accounted for in all models, and the effects of metaphylaxis treatment and gender (both with 2  
28 levels) were forced into all models. Logistic regression was employed to analyze proportional  
29 outcomes.<sup>y</sup> Random effects of calf identification number, pen, and lot were accounted for in the  
30 analysis. For the Fall 2007 trial, we measured the association between BVDV antibody titers at

1 arrival on the subsequent BVDV viremic status at revaccination, morbidity risk, mortality risk,  
2 and feed performance. In addition, we evaluated potential associations between BVDV viremia  
3 at revaccination and morbidity risk, mortality risk, and feed performance. For the Spring 2008  
4 study, we estimated the risk of viremia based on treatment group (vaccinate or control) and day  
5 of sample collection. A subset of the data consisting of vaccinated calves in the Spring 2008 trial  
6 that were rt-PCR and sequence positive on day 7 (n=13) was created to evaluate the probability  
7 of clearing the virus by day 21 post-vaccination and potential associations between arrival  
8 BVDV titers and subsequent duration of BVDV viremia.

## 9 **Results**

### 10 *Fall 2007 Trial*

11 One hundred eighty bulls and one hundred thirteen steers averaging 218 kilograms (kg)  
12 were included in the three loads of calves comprising the Fall 2007 trial. Cattle were at the  
13 growing facility for 43 (load one) or 41 days (loads two and three). All arrival ACE (ear biopsy)  
14 and PCR tests (plasma) were negative for BVDV. At arrival, 15.1% (n=44) of the calves were  
15 sero-negative to both BVDV genotypes, 34.2% (n=100) were sero-positive to BVDV type one  
16 but not type two, 3.4% (n=10) were sero-positive to type two but not type one, and 47.3%  
17 (n=138) were sero-positive to both genotypes.

18 The overall morbidity risk, retreatment risk, and mortality risk for the trial was 51%  
19 (149/293), 48% (71/149), 9.5% (28/293), respectively. One calf died the day of arrival (Load 3)  
20 but prior to vaccine administration. The BVDV status of this calf was not determined. In  
21 addition to gross necropsy, a diagnostic workup consisting of fluorescent antibody, virus  
22 isolation, and bacteriology tests were performed on 24 of the 28 dead calves. Of these 24 calves,  
23 BVDV was isolated in 12 individuals from various tissues.

24 At revaccination, 28.8% (81/281) of the remaining population were PCR positive for  
25 BVDV. No statistical differences (P=0.7) were detected in the percentage of BVDV positive  
26 cattle among pens within the facility. Cattle found to be viremic with BVDV at revaccination  
27 (Day 10 post-vaccination) were at a higher risk of being treated for BRD, death, and treated  
28 significantly earlier in the feeding phase compared to non-viremic cattle (Table 2.1). The overall  
29 average daily gain (ADG) of viremic cattle was significantly less compared to non-viremic cattle  
30 (Table 2.1). Cattle viremic with BVDV at the first BRD treatment were observed to be at greater

1 risk ( $P < 0.05$ ) of being retreated for BRD (0.54; 95% Confidence Interval [95% CI], 0.39 – 0.67)  
2 compared to non-viremic cattle (0.34; 95% CI, 0.23 – 0.47) at first BRD treatment. However, no  
3 association ( $P > 0.05$ ) was observed with regard to the risk of mortality at the first BRD treatment  
4 among viremic (0.14; 95% CI, 0.08 – 0.24) and non-viremic (0.15; 95% CI, 0.09 – 0.25) cattle.

5 The BVDV antibody status at arrival tended to be associated with BVDV status by PCR  
6 at revaccination ( $P = 0.08$ ). The probability of testing positive to BVDV on day 10 by PCR for  
7 each BVDV antibody category is shown in Table 2.2. However, no significant associations were  
8 detected between arrival antibody status for BVDV and morbidity or mortality risk, the number  
9 of days on feed prior to first treatment for BRD, or overall ADG.

### 10 *Spring Trial 2008*

11 Twenty two mixed-breed beef steers averaging 193 kg were enrolled in the Spring 2008  
12 study. All arrival ACE (ear biopsies) and PCR tests (serum) were negative for BVDV. At  
13 arrival, 43.8% (7/16) and 33.3% (2/6) of the vaccinated and control cattle, respectively,  
14 possessed antibodies ( $\geq 1:4$ ) to both BVDV genotypes. In the vaccinated animals, 81% (13/16)  
15 were PCR positive for BVDV seven days after vaccination, while 63% (10/16) were PCR  
16 positive on days 14 and 21, post-vaccination. In non-vaccinated cattle, 17% (1/6) were PCR  
17 positive on days seven and 14 post-vaccination, while 50% (3/6) were PCR positive 21 days,  
18 post-vaccination. Throughout the entire 21 day study, 87.5% (14/16) of the vaccinates and 50%  
19 (3/6) of the control calves became PCR BVDV positive at least one time post-vaccination. A  
20 morbidity risk of 4.5% (1/22) for bovine respiratory disease (BRD) was observed during the trial.  
21 No death loss was observed and ADG was not measured in this study.

22 Sequencing of RNA confirmed that the positive PCR tests resulted from the contaminant  
23 BVDV originally identified by KSU during the Fall 2007 study. A discrepancy between positive  
24 PCR and negative genetic sequence results from individual calves were observed on day 7 ( $n = 1$ ),  
25 day 14 ( $n = 1$ ), and day 21 ( $n = 8$ ). In addition, four calves on day 21 had positive PCR results but  
26 inconclusive sequence data. The remaining PCR and RNA sequence outcomes were identical.

27 The model-adjusted probability of testing PCR positive throughout the study tended  
28 ( $p = 0.06$ ) to be higher in vaccinates (0.70; CI, 0.46 – 0.86) compared to non-vaccinated controls  
29 (0.26; CI, 0.06 – 0.67). Likewise, the probability of testing BVDV positive by RNA sequencing  
30 was significantly different between vaccinates (0.41; CI, 0.21 – 0.64) and controls (0.024; CI,



1 0.002 – 0.23). Across both treatment groups, the probability of testing positive to BVDV by  
2 RNA sequencing during the study was significantly different (Table 2.3).

3 The probability of individual calves developing antibodies to BVDV types 1 and 2 over  
4 time in both treatment groups significantly increased throughout the course of the study (Table  
5 2.4). No statistical differences were observed in the risk of developing BVDV antibody titers  
6 between days 0 and seven and days 14 and 21, post-vaccination (Table 2.4). The risk of  
7 becoming antibody positive to BVDV type 1 did not differ between treatment groups. However,  
8 vaccinated calves tended ( $p=0.08$ ) to have a higher probability of becoming BVDV type two  
9 antibody positive compared to non-vaccinated calves throughout the study.

10 A subset of the data was created to evaluate the potential association between arrival  
11 BVDV antibody status and the risk of clearing the BVDV infection by day 21, post-vaccination.  
12 These data included only vaccinated calves that became PCR positive and sequence positive for  
13 BVDV on day seven after vaccination ( $n=13$ ). Cattle antibody positive at arrival to both BVDV  
14 genotypes (4/13) tended ( $p=0.07$ ) to possess a higher probability of being BVDV negative (0.75;  
15 CI, 0.19 – 0.97) on day 21 relative to antibody negative cattle (9/13) (0.11; CI, 0.01 – 0.56)  
16 based upon PCR results. However, based upon genetic sequence data, no differences were  
17 observed between sero-status when comparing the risk of clearing the BVDV infection by day  
18 21.

## 19 **Discussion**

20 This retrospective analysis illustrates that administration of vaccine contaminated with  
21 BVDV was associated with calves testing PCR and sequence positive for BVDV (Spring 2008)  
22 and the subsequent viremia was associated with negative health and performance outcomes (Fall  
23 2007). During these studies, we were unaware that the MLV vaccine administered to cattle was  
24 contaminated with a NCP type II BVDV. Therefore, the observations of high morbidity and  
25 mortality risks, extensive BVDV viremia post-vaccination, and isolation of BVDV in dead cattle  
26 were not easily explained given the biosecurity of the cattle facility and the negative BVDV  
27 status of all cattle at arrival in both studies. However, subsequent knowledge of the extraneous  
28 BVDV isolate contained in the vaccine provided the opportunity to retrospectively evaluate  
29 potential associations between the contaminated vaccine and the outcomes witnessed in both  
30 studies.

1 Prior reports investigating the administration of BVDV contaminated vaccine to cattle  
2 suggest that health and performance effects may vary. Barkema et al (2001) described multiple  
3 episodes of type two BVDV infection following administration of a BVDV contaminated bovine  
4 herpesvirus 1 (BHV-1) vaccine in the Netherlands.<sup>210</sup> Morbidity risk on five affected farms was  
5 observed to be greater than 70% while another farm was free of clinical illness.<sup>210</sup> Likewise,  
6 Falcone et al (2003) described a NCP type two BVDV isolate, recovered from BHV-1 vaccine,  
7 which was inoculated into four three-month old calves.<sup>211</sup> Three of the calves, sero-negative to  
8 BVDV at the time of inoculation, developed disease four to six days post-inoculation, and the  
9 BVDV isolated from these calves revealed 100% homology with the isolate recovered from the  
10 vaccine.<sup>211</sup>

11 Despite administration of the contaminated vaccine to cattle in both studies, cumulative  
12 incidence risks for morbidity and mortality among cattle comprising the Fall 2007 trial (51% and  
13 9.5%, respectively) was relatively high compared to the Spring 2008 study in which virtually no  
14 adverse health effects were observed. Potential explanations of disparity in disease risk between  
15 trials include differences in procurement (e.g. location, distance traveled, health history),  
16 management of cattle, and season of arrival to the research facility. Cattle from the Spring 2008  
17 trial were obtained through a local livestock auction market (located approximately 60 miles  
18 north of Manhattan, KS) and were on feed for 21 days compared to 42 days for calves in the Fall  
19 2007 study. Conversely, cattle composing the Fall 2007 trial originated in the southeastern  
20 sector of the United States, were assembled through multiple auction markets, and were  
21 transported from Tennessee to Kansas. Only 22 head comprised the Spring 2008 study  
22 compared to 293 in the Fall 2007 trial, potentially minimizing the extent of commingling  
23 occurring among cattle from different origins. The sample population from the Fall 2007 study  
24 was potentially subjected to a greater degree of stress, a higher level of microbial exposure, and a  
25 greater compromise of overall immunity compared to the Spring 2008 cattle. Therefore, despite  
26 the fact that the same BVDV isolate was administered to both cattle populations, procurement  
27 and management disparities may have contributed to the dissimilarities observed in health  
28 parameters between the two studies.

29 In the Fall 2007 trial, BVDV was not observed in cattle upon arrival; however, a  
30 moderate percentage of calves (28.8%) were subsequently BVDV PCR positive at revaccination.  
31 Given that commingling did not occur among cattle from different loads, a lack of difference

1 among the percentage of BVDV-positive cattle at revaccination across all pens suggests that  
2 there were not differential exposures among loads or pens. However, given that the population  
3 was negative for BVDV at arrival and the strict biosecurity of the facility prevented random  
4 sources of exposure, the presence of BVDV at revaccination was likely induced by the  
5 administration of the vaccine to all calves at arrival. The subsequent viremia at revaccination  
6 (day 10) was associated with negative health and performance outcomes. This is in agreement  
7 with other authors who observed that the presence of viremia in cattle inoculated with BVDV  
8 was associated with elevated adverse health effects.<sup>171,212,213</sup>

9 Despite the associations between BVDV viremia on day 10 post-vaccination and health  
10 outcomes in the Fall 2007 trial, it should be stressed that there was no negative controls in this  
11 study. Therefore, this data cannot provide an estimate of the direct effect of vaccine  
12 administration on health and performance. Although these findings illustrate an association  
13 between BVDV viremia at revaccination (day 10) and negative health and performance  
14 outcomes, they do not necessarily indicate causality.

15 Surprisingly, the presence of BVDV antibody titers at arrival did not significantly impact  
16 health or performance outcomes in either study. This is in contrast with a previous study that  
17 reported cattle with BVDV titers at arrival were associated with decreased odds of being treated  
18 for BRD,<sup>214</sup> however, the cattle in that study were presumably not exposed to BVDV in the  
19 manner witnessed in the present study. In the Fall 2007 study, BVDV antibody status at arrival  
20 tended to be associated with BVDV viremia on day 10. Previous studies report that calves  
21 vaccinated with a type I BVDV MLV vaccine and subsequently infected with a type II BVDV  
22 isolate were protected from viremia and clinical disease.<sup>170,171</sup> The present scenario is unique as  
23 cattle in both studies were administered the vaccine and the BVDV challenge at the same time  
24 thereby eliminating the opportunity for cattle to adequately respond to the vaccine prior to  
25 BVDV infection.

26 The BVDV PCR results cannot be directly compared between the Fall 2007 and Spring  
27 2008 studies. The same PCR test method was utilized in both trials; however, three tests were  
28 performed in parallel in the Spring 2008 study compared to only one test (at day 10) in the Fall  
29 2007 trial. Therefore, a positive BVDV diagnosis was classified by at least one of three positive  
30 PCR tests in the Spring 2008 trial. The integration of parallel interpretation likely increased the  
31 diagnostic sensitivity of detecting BVDV viremic calves in the Spring 2008 trial as compared to

1 the Fall 2007 study, but may have resulted in a reduction of diagnostic specificity.<sup>215</sup> This  
2 method of interpretation was employed for the retrospective analysis in the Spring 2008 study  
3 due to the inclusion of serial sample collection over time and confirmed genetic sequence  
4 homology between BVDV isolated from cattle and the vaccine contaminant. Maximizing the  
5 diagnostic sensitivity of the test increases apparent prevalence of viremic cattle at each  
6 respective time point. This information is practical as it provides a potential “worst case  
7 scenario” of the prevalence of cattle at risk of shedding the BVDV contaminant post-vaccination.  
8 Conversely, the Fall 2007 trial did not provide sequential points of testing and sample quality  
9 was not sufficient to allow adequate interpretation of genetic sequencing results. Therefore, it is  
10 possible that the prevalence for BVDV viremia at revaccination and at the first BRD treatment is  
11 underestimated in the Fall 2007 trial.

12 Vaccinated cattle in the Spring 2008 study tended to be at greater risk for viremia at any  
13 point during the study compared to non-vaccinated penmates. However, 50% of the non-  
14 vaccinated calves became viremic with the extraneous BVDV at least once during the study.  
15 This suggests that vaccinated cattle were shedding the BVDV contaminant to non-vaccinated  
16 cattle housed in the same pen. The onset of horizontal transmission appears to be somewhat  
17 rapid as a control calf was PCR positive to BVDV by day seven, post-vaccination. No additional  
18 samples were collected between days zero (arrival) and seven; therefore, an exact time lapse  
19 between shedding of vaccinated cattle and detectable viremia in negative controls cannot be  
20 determined. These findings are similar to Fulton et al (2005) who studied the rate of BVDV  
21 transmission in 10 pens of healthy calves.<sup>66</sup> In that study, one PI calf was housed with non-PI  
22 cattle in each pen for the duration of the study (35 days).<sup>66</sup> Non-PI calves became viremic  
23 between days seven and 21, post-exposure.<sup>66</sup> In our study, PI cattle were not present; however, a  
24 unique class of transiently infected (TI) cattle was produced due to the administration of the  
25 same extraneous BVDV isolate, at approximately the same time.

26 No statistical differences were observed in the probability of testing positive for BVDV  
27 on each day of sample collection for the PCR test in the Spring 2008 study. Conversely,  
28 vaccinated cattle displayed an increasing probability of being positive for BVDV on any of the  
29 three testing days based on genetic sequencing. These data are likely attributed to differences in  
30 diagnostic sensitivity between the two tests and suggest that the viral load circulating in the pen  
31 was declining at this point in time. The extraneous NCP type II BVDV was still present on day

1 21, post-vaccination, as diagnosed by PCR (n=13) and confirmed with genetic sequencing (n=1).  
2 However, we cannot be certain of the duration of infection or the probability of new infections  
3 arising since samples were not collected at later time points. Therefore, regardless of testing  
4 method, our results suggest that cattle administered the contaminated vaccine are potentially  
5 viremic (and potentially infectious) for at least three weeks post-vaccination. One limitation of  
6 these data is the length of time cattle were monitored in the spring 2008 study. However, the  
7 study was designed based on the assumption that all cattle would have been non-viremic by 21  
8 days. Other authors have reported BVDV vaccine viremia durations significantly shorter than 21  
9 days.<sup>160,193,216-218</sup>

## 10 **Conclusion**

11 Cattle administered MLV vaccine contaminated with a NCP type II BVDV isolate  
12 developed a transient viremia still present in one of 16 calves 21 days post-vaccination. BVDV  
13 viremia 10 days post-vaccination, as diagnosed by PCR and presumably attributed to the BVDV  
14 contaminant, was a risk factor for BRD morbidity, BRD retreatment, mortality, and reduced  
15 weight gain. Positive antibody titers for BVDV at arrival did not significantly affect  
16 performance of cattle vaccinated with the contaminated vaccine. Additionally, the viral  
17 contaminant was observed to have been shed from vaccinated cattle to unvaccinated controls  
18 housed in the same pen. Variation in health outcomes was witnessed across both studies.

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

1 **Table 3.1: Model-adjusted<sup>1</sup> means and 95% confidence intervals for health measures**  
 2 **(morbidity, repull, mortality, and days on feed (DOF) at first pull) and average daily gain**  
 3 **(ADG) by BVDV status (by PCR) at revaccination for the Fall 2007 study.**

Revaccination BVDV Status	Morbidity (%)	Repull (%)	Mortality (%)	ADG (lbs)	DOF at 1 <sup>st</sup> Pull (days)
Positive	72.3 (60.7 – 80.6) <sup>a</sup>	69.8 (55.7 – 81) <sup>a</sup>	14.5 (8 – 24.9) <sup>a</sup>	1.7 (1.5 – 1.9) <sup>a</sup>	8.5 (7.1 – 9.9) <sup>a</sup>
Negative	42.8 (35.5 – 50.6) <sup>b</sup>	20.7 (12.8 – 31.7) <sup>b</sup>	2.9 (1.3 – 6.4) <sup>b</sup>	2.5 (2.4 – 2.7) <sup>b</sup>	10 (8.8 – 11.3) <sup>b</sup>

4

5 <sup>a, b</sup>Means in columns with different superscripts are significantly different (P<0.05)

6 <sup>1</sup> From mixed models accounting for viremia status at revaccination and arrival metaphylactic  
 7 treatment as fixed effects. Only the results of the effect of interest (risk of viremia) are reported.

8

9

1 **Table 3.2: Model-adjusted probability and 95% confidence intervals measuring the risk of**  
 2 **individual cattle testing positive for BVDV on day 10 based on BVDV antibody status<sup>1</sup> at**  
 3 **arrival.**

BVDV Antibody Category	Probability of being BVDV Positive on Day 10 by PCR	95% Confidence Interval
No BVDV antibody titers to either genotype	0.53 <sup>a</sup>	0.30 – 0.74
BVDV antibody titers to type II, not type I	0.54 <sup>a</sup>	0.12 – 0.91
BVDV antibody titers to type I, not type II	0.50 <sup>a</sup>	0.34 – 0.65
BVDV antibody titers to both genotypes	0.28 <sup>b</sup>	0.17 – 0.42

4 <sup>a, b</sup>Means in columns with different superscripts are significantly different (P<0.05)

5 <sup>1</sup>Based on least squared means and 95% confidence intervals from mixed models accounting for  
 6 BVDV antibody status at arrival and metaphylactic treatment as fixed effects. Only the results of  
 7 the effect of interest (risk of BVDV viremia) are reported.

1 **Table 3.3: Risk<sup>1</sup> and 95% confidence intervals for testing positive for BVDV by genetic**  
 2 **sequence on days 7, 14, and 21, post-vaccination of cattle in Spring 2008 study (n=22).**

	Day 7	Day 14	Day 21
Risk of positive PCR	0.48 (0.20 – 0.78) <sup>a</sup>	0.36 (0.14 – 0.67) <sup>a</sup>	0.56 (0.32 – 0.78) <sup>a</sup>
Risk of positive genetic sequence	0.39 (0.13 – 0.72) <sup>a</sup>	0.22 (0.06 – 0.56) <sup>a</sup>	0.01 (0.001 – 0.13) <sup>b</sup>

3 <sup>a, b</sup>Means in rows with different superscripts are significantly different (P<0.05)

4 <sup>1</sup>Based on least squared means and 95% confidence intervals from mixed models accounting for  
 5 viremia status and treatment group at each day of sample collection. Only the results of the  
 6 effect of interest (risk of viremia) are reported.



1 **Table 3.4: Risk<sup>1</sup> and 95% confidence intervals for developing titers to BVDV genotypes 1**  
 2 **and 2 on days 7, 14, and 21, post-vaccination of cattle in Spring 2008 study (n=22).**

	Day 0	Day 7	Day 14	Day 21
BVDV type 1 (%)	0.30 (0.08- 0.69) <sup>a</sup>	0.38 (0.11 – 0.76) <sup>a</sup>	0.93 (0.68 – 0.99) <sup>b</sup>	0.96 (0.75 – 0.99) <sup>b</sup>
BVDV type 2 (%)	0.14 (0.02 – 0.59) <sup>a</sup>	0.14 (0.02 – 0.59) <sup>a</sup>	0.85 (0.38 – 0.98) <sup>b</sup>	0.96 (0.67 – 0.99) <sup>b</sup>

3

4 <sup>a, b</sup>Means in rows with different superscripts are significantly different (P<0.05)

5 <sup>1</sup>Based on least squared means and 95% confidence intervals from mixed models accounting for  
 6 a titer status and treatment group at each day of sample collection. Only the results of the effect  
 7 of interest (risk of a positive titer) are reported.

# **CHAPTER 4 - Evidence of viral transmission and nasal shedding among beef calves exposed to a calf persistently infected with bovine viral diarrhea virus**

## **Introduction**

Bovine viral diarrhea virus (BVDV) possesses world-wide prevalence and is a significant source of production loss in beef and dairy production systems.<sup>1-9</sup> The viral reservoir is believed to be cattle persistently infected (PI) with BVDV which are lifelong shedders of virus thereby increasing the efficiency of viral transmission compared to transiently infected (TI) cattle.<sup>2-4,10</sup> Cattle PI with BVDV have previously been observed to possess serum viremias of  $10^4 - 10^6$  cell culture infectious dose<sub>50</sub> (CCID-50)/ml.<sup>2,3,11</sup> Serum BVDV levels also have been observed to approximate the viral concentrations in nasal secretions, urine, feces, and uterine secretions.<sup>2,12</sup> Primary BVDV prevention and control strategies are targeted toward increasing population immunity through vaccination or decreasing pathogen exposure by removing PI cattle and prohibiting their entry into the herd.<sup>13</sup> Consequently, several diagnostic testing strategies,<sup>14-16</sup> and vaccination programs,<sup>17-19</sup> and biosecurity protocols<sup>7,20,21</sup> have been suggested to minimize the impact of BVDV.

The onset, duration, and clinical effects among antibody-negative non-PI cattle when experimentally (intranasal or intramuscular inoculation) or naturally infected (by means of PI exposure) with BVDV have been studied.<sup>22-26</sup> These findings suggest that BVDV antibody-negative cattle transiently infected (TI) with BVDV are observed to be viremic (buffy coat and serum) between days 3-15 post-exposure,<sup>23,24</sup> display widespread viral distribution to organ systems,<sup>23</sup> may shed virus in nasal secretions,<sup>26</sup> and display variable clinical outcomes.<sup>22,24</sup>

Despite the knowledge gained from the aforementioned research, few studies have implemented models utilizing PI cattle as the mode of infection among clusters of non-PI cattle possessing diverse levels of BVDV antibody titers typical of cattle in commercial production operations.<sup>27-32</sup> Determination of the time necessary for a non-PI calf to develop BVDV infection post-exposure to a PI calf is important as this may influence design of control programs. The duration of the transient infection and the capability of these TI animals to shed

BVDV may also influence the magnitude of detrimental health impacts of BVDV in the population.

A TI diagnosis has traditionally been performed by virus isolation (VI) on serum or buffy coat. However, VI on buffy coat has been observed to provide more accurate results when compared to serum and is currently regarded as the recommended gold standard for BVDV diagnosis.<sup>10,27,28</sup> Despite these recommendations, the logistical aspects of capturing the buffy coat fraction from a whole blood specimen may preclude practicing veterinarians from collecting buffy coat from field samples. Serum provides the practitioners with a more convenient and practical alternative to buffy coat.

The objectives of this study were two-fold: 1) to describe the frequency and time to onset of viral transmission between a PI calf and a non-PI study population in a production setting based on serum VI, and 2) to characterize the transient BVDV infection in the non-PI study population based on duration of positive serum viremic status and potential for shedding the virus in nasal secretions. A greater understanding of viral transmission in a beef cattle production setting facilitates knowledge-based modifications to current BVDV preventative and control programs.

## **Materials and Methods**

All animals were handled in accordance with a protocol approved by the Kansas State University Animal Care and Use Committee. The two studies were performed by introducing a known PI calf into a confirmed BVDV antigen-negative population of cattle. The exposure period in each study lasted 21 days and multiple samples were collected throughout the studies.

In study one, twelve mixed-breed beef steer calves, averaging 184.2 kg, were procured through a livestock auction market in northeast Kansas. The health history of all calves was unknown at the time of purchase. Fifteen days prior to PI calf introduction (day -15), all calves were delivered to a Kansas State University (KSU) facility consisting of an open-air dirt-floor pen with a total area of 544 m<sup>2</sup> (5859 ft<sup>2</sup>). Twenty-four hours after arrival (day -14), all calves received a two milliliter (mL) multi-valent clostridial vaccine<sup>a</sup>, an injectable dewormer<sup>b</sup>, and a topical insecticide<sup>c</sup>. Additionally, a standard triangular ear biopsy, measuring approximately one cm<sup>2</sup>, was collected by a commercial ear notch device<sup>d</sup>. Blood was collected in one six mL red top clot tube and one four mL EDTA tube from each individual by jugular venipuncture. A

second blood sample collected in the same manner was attained on day -4. In study two, 15 mixed breed beef bull calves were procured through the same industry channels and housed in the same pen as calves in study one. On day -16, all bulls were surgically dehorned and castrated by standard industry approved methods and received a two mL multi-valent clostridial vaccine<sup>a</sup>, an injectable dewormer<sup>b</sup>, and a topical insecticide<sup>c</sup>. Additionally, a standard ear notch biopsy, measuring approximately one cm<sup>2</sup>, was collected by a commercial ear notch device<sup>d</sup>. Blood was collected in the same manner as in study one. A second blood sample was collected on day -6. In both studies, the inclusion criteria for the BVDV-negative population was a negative antigen capture ELISA (ACE) test on ear tissue (days -14 and -16 in studies one and two, respectively) and two negative serum VI tests (days -14 and -4, and days -16 and -6, in studies one and two, respectively). All tests were performed at the Kansas State Veterinary Diagnostic Laboratory (KSVDL).

In both studies, the inclusion criteria for the BVDV PI animal included two positive ACE (skin) and VI tests (serum) two weeks apart by the KSVDL. In study one, one mixed breed beef steer calf was identified as PI during a previous KSU research study. This calf weighed approximately 181.4 kg on day 0 of study one and had been isolated in an off-site facility prior to introduction. In study two, the PI calf had been identified as potentially PI by a commercial feedlot in central Kansas. This calf weighed approximately 363.6 kg on day 0 of study 2 and had remained at the aforementioned feedlot prior to introduction to the non-PI study population. Genotyping and subtyping analysis of both PI calves was completed by the KSVDL.

On day 0 of both studies, the PI calf was commingled with the non-PI population. Blood was collected in two five mL red top clot tubes and two four mL EDTA tubes from each individual calf by jugular venipuncture. One ear biopsy measuring approximately one cm<sup>2</sup> was collected from each individual calf and placed in sterile plastic tubes. One sterile nasal swab<sup>e</sup> was inserted approximately 10 cm (four inches) into the nostril, rested against the mucosal surface, and gently rubbed against the nasal mucosa in a circumferential pattern five times. Blood was collected in the same manner every other day for the duration of both studies. Due to the frequency of jugular venipuncture, both jugular grooves were clipped prior to day 0, the side of blood collection was alternated between each timepoint, and the site of injection was cleansed with chlorhexidine and alcohol prior to needle insertion to minimize the risk of phlebitis. Ear

biopsies and nasals swabs were collected every four days for the duration of both studies. Each PI calf remained with its respective population for 21 days after introduction.

Within one hour of each sample collection, blood tubes were centrifuged at 2350 rcf (g) for 10 minutes and serum, plasma, and buffy coat were harvested in a traditional manner. Buffy coat samples in study one failed to be collected until day 12 of the study; however, buffy coat was collected for the entirety of study two. All specimens (serum, plasma, buffy coat, skin biopsies, and nasal swab samples) were stored at -80°C until further diagnostics were performed.

Throughout both studies, animal caretakers offered a total mixed ration equating to 1.5% body weight (DMI) twice daily while grass hay and fresh water was offered *ad libitum*. All calves were observed twice daily by a veterinarian to monitor individual health status. Rectal temperatures and further examination of individuals suspected of clinical disease were attained prior to treatment. Animals displaying clinical signs of respiratory disease and rectal temperatures  $\geq 40^{\circ}$  C were classified as individuals experiencing bovine respiratory disease (BRD) and were treated following a predesigned treatment protocol. The treatment protocol dictated that cattle meeting the treatment criteria for BRD were treated with tulathromycin<sup>e</sup> (2.5 mg/kg SC), florfenicol<sup>f</sup> (40mg/kg, SC), and oxytetracycline<sup>g</sup> (20mg/kg, SC) for the first, second, and third treatments, respectively. The pen was idle for approximately two months between the two studies and during this time the watering devices and feed bunks were cleansed and disinfected and the pen floor was scraped and all organic material was removed.

On the last day of both studies (day 21), the PI calf was removed from the pen. The remaining non-PI calves were maintained at the study site until negative VI tests (on serum) were confirmed on all non-PI cattle. Upon a cumulative VI negative status, all non-PI animals left the facility on the same day.

### ***Serology***

In both studies, serum neutralization assays for type one, type two, and the homologous PI BVDV were performed on serum at the KSVDL using the assay format as previously described.<sup>33</sup> Antibody titers were quantified from serum samples from all calves from both studies on days 0 and 21.

## ***Virus Isolation***

All quantitative VI attempts from both studies were conducted by student workers and completed in the KSVDL. Samples for quantitative VI were subjected to four ten-fold serial dilutions and two hundred  $\mu\text{L}$ s of each respective sample/dilution were then added to freshly seeded embryonic bovine lung (EBL) cell cultures in 96 well micro-titer plates. The assay plates were then incubated for 48 hours at  $37^{\circ}\text{C}$  in 4.5%  $\text{CO}_2$ . After incubation the media in each plate was discarded and the cells were washed with FA-PBS and fixed in an 80% aqueous acetone solution for 10 minutes. After discarding the acetone, the plates were allowed to dry at room temperature. An indirect fluorescent antibody test was used to detect BVD infected cells. Fifty  $\mu\text{L}$ s of the primary anti-BVDV monoclonal antibody (D89)<sup>34</sup> was added to each well and allowed to incubate for 30 minutes. The primary antibody was then discarded and the plate washed with FA-PBS and fifty  $\mu\text{L}$ s of the FITC labeled secondary<sup>i</sup> was added to each well and allowed to incubate for 30 minutes. After incubation the secondary antibody was then discarded and the plate was washed with FA-PBS and fifty  $\mu\text{L}$ s of 50% glycerol were added to each well prior to reading the plates. The cell-culture infectious dose (CCID-50) was calculated by the method of Spearman and Karber as previously described method.<sup>35</sup>

## ***Real-time Reverse Transcriptase Polymerase Chain Reaction***

*RNA extraction of serum samples* – One hundred  $\mu\text{L}$ s of serum from all calves were utilized for extraction of viral RNA for use in quantitative real-time RT-PCR (*rRT-PCR*) analysis. Magnetic bead based nucleic acid isolation method was used to extract Viral RNA using a commercial isolation kit<sup>j</sup> by following manufactures instructions. A commercial processor and protocol<sup>k,l</sup> was utilized for RNA extraction with an elution volume of 90  $\mu\text{L}$ . The RNA from the samples was stored at  $-80^{\circ}\text{C}$  until use.

*RNA extraction of nasal swab samples* – The nasal swab samples were vortexed at low speed for 10 seconds. One hundred  $\mu\text{L}$ s of the sample was then utilized for RNA extraction by following the procedure as described above.

*Quantitative real-time RT-PCR (rRT-PCR) analysis* – The *rRT-PCR* assay was performed using a commercial test kit<sup>m</sup> to identify BVDV RNA in the serum samples. The *qRT-PCR* analysis was performed in a 25  $\mu\text{L}$  reaction volume containing 2X RT-PCR buffer (12.5  $\mu\text{L}$ ), 25X BVDV primer probe mix (1  $\mu\text{L}$ ), 25X RT-PCR Enzyme Mix (1  $\mu\text{L}$ ), sample template

(8  $\mu$ L) and nuclease free water (2.5  $\mu$ L). The PCR analysis also included appropriate positive and negative controls. Commercial templates<sup>n</sup> served as positive controls for the PCR reaction. A reaction containing all the reagents but no template was also included to serve as a negative control. The assay was performed in a commercial system<sup>o</sup> under standard run mode. Thermal profile for the PCR reaction included an initial cDNA synthesis step of 10 min at 45 °C followed by RT inactivation and initial denaturation step for 10 min at 95 °C, then 40 cycles of 95 °C for 15 sec, and 60 °C for 45 seconds. The *r*RT-PCR data analysis was performed with the help of commercial software<sup>p</sup>. Prior to the data analysis, the *r*RT-PCR run was validated by verifying the cycle threshold (Ct) values of positive and negative controls reactions (BVDV RNA target, Ct  $\leq$  28; Xeno RNA control target, Ct  $\leq$  31; and no-template control reaction, no signal or Ct = 40). The results were then interpreted based on Ct distributions per the manufacturer's recommendations<sup>l</sup>, where Ct  $\leq$  38 were considered positive, Ct values between 38-40 were considered suspect results, and Ct  $>$  40 were considered negative.

In both studies, the case definition for an animal at risk for becoming *r*RT-PCR positive included the timeframe from when the PI calf was introduced to the BVDV negative subpopulation (day 0) up until a positive test result was first confirmed among individual cattle. Therefore, when/if the calf displayed a positive PCR outcome, that respective calf was no longer at risk for positive PCR status. Given that serum was collected every other day, the duration of positive PCR status was defined from the initial day of positive PCR status to the day prior to the first negative PCR test. For example, if a calf had positive serum PCR results on days 8 and 10 but tested negative on day 12, it would be classified as PCR positive for a duration of three days (days 8 – 11).

### ***Statistical Analysis***

The individual calf was the experimental unit throughout the data analysis. Descriptive statistics and graphical procedures for all measured variables were obtained by a commercial software package<sup>q</sup>. Assessment for normality among continuous distributions (day of TI onset and duration of TI) was performed by visual assessment as well as by formal statistical methods<sup>r</sup>. As per standard practice, if continuous data were determined not to be normally distributed, the median value and range of data (not the average and standard error) was implemented as the necessary point estimate and description of data variation, respectively.

## Results

All screening phase (pre day 0) ear biopsies and serum samples from the non-PI calves in both studies were negative for BVDV. The status of both PI animals was confirmed through ACE (on ear tissue) and VI (on serum). In study one, a cumulative morbidity risk of 8.3% (1/12) was observed among the non-PI population while no morbidity (0%) was observed in study two. At arrival (pre day 0) 25% (n=3) of non-PI calves enrolled in study one were negative for type one and type two BVDV antibodies as well as antibodies to the homologous strain infecting the PI calf. The remaining non-PI population (n=9) possessed antibodies to both BVDV genotypes as well as the homologous PI strain (Table 1). On day 21 post-exposure, 41.8% (n=5), 16.7% (n=2), and 100% (n=12) of the non-PI calves displayed a four-fold rise in serum antibodies to BVDV type one, type two, and the homologous PI strain, respectively. At arrival, 6.7% (n=1) of non-PI calves enrolled in study two were negative for both type one and type two BVDV antibodies as well as antibodies to the homologous strain possessed by the PI calf (Table 1). Of the remaining non-PI population in study two, 40% (n=6) possessed antibodies to type one, type two, and the homologous PI virus, 20% (n=3) possessed antibodies to both BVDV genotypes but not the homologous PI virus, and 33% (n=5) possessed only BVDV type two titers. By day 21 of study two, 26.7% (n=4), 26.7% (n=4), and 60% (n=9) of the non-PI calves displayed a four-fold rise in serum antibodies to BVDV type one, type two, and the homologous PI strain, respectively.

Virus isolation performed on serum did not identify BVDV among the non-PI calves at any sampling point throughout the duration of both studies. Serum VI tests from both PI calves in each respective study were positive on every day of sample collection with a range of serum BVDV concentrations of  $10^{1.70} - 10^{3.70}$  CCID-50/mL of serum (Figure 1). Similarly, VI performed on nasal swabs did not identify BVDV among the non-PI population; however, samples from study two displayed a relatively high degree of contamination. Conversely, nasal swabs collected from the PI calves were positive by VI on days 16 and 20 in study one and days 0, 4, 8, 12, 16, and 21 in study two.

Due to the inability of VI to isolate BVDV in serum in either study, BVDV was successfully isolated from one buffy coat sample (the classical gold standard) of one randomly selected TI calf for genotyping and sequencing. As expected, virus was detected and genetic sequencing determined that the homologous BVDV from each respective PI calf were



genetically different; however, each was classified as BVDV type 1b. Additionally, isolated BVDV from the aforementioned TI cattle was homogenous with virus isolated from each respective PI calf in both studies (data not shown).

Given the PCR manufacturer's recommendation of Ct values indicating positive test results ( $Ct \leq 38$ ), 83.3% (n=10) and 80% (n=12) of the non-PI calves were serum PCR positive for BVDV nucleic acid at least once during studies one and two, respectively (Table 1). The remaining calves in studies one (n=2) and two (n=3) were found to be PCR negative ( $Ct > 40$ ) at all collection points. However, 80% (n=4) of these PCR negative non-PI calves displayed a four-fold increase in serum antibodies to the homologous BVDV PI strain in their respective study indicating that they were indeed TI.

Cumulatively, across both studies, 77.8% (14/18) and 88.9% (8/9) of non-PI calves with and without antibodies to the homologous PI strain of BVDV, respectively, became PCR positive to BVDV at some point during their respective study (Table 1). The day of initial positive PCR status and subsequent duration were both determined not to be normally distributed. Therefore, across both studies, the median day of the initial positive BVDV PCR diagnosis was day 10 (range: 6 – 21 days) post-exposure to the PI calf while the median duration of positive BVDV PCR status was 3 days (range; 1 – 9 days) (Table 1).

In studies one and two, respectively, nasal swabs from 66.7% (8/12) and 80% (12/15) of non-PI cattle were found to be PCR-positive for BVDV at least once (Table 1). Among the non-PI calves that tested PCR positive on serum for BVDV, 70% (7/10) of calves in study one and 91.7% (11/12) of calves in study two had positive nasal swabs. Due to the infrequency of nasal swab collection, the time of onset and duration of PCR positive status of nasal swabs were not estimated.

## **Discussion**

The results of this study indicate that BVDV is easily and rapidly transmitted from an individual recognized as PI with BVDV to non-PI individuals demonstrated by *r*RT-PCR and confirmed by seroconversion but not necessarily by VI from serum or nasal swabs. The experimental method demonstrated in this study which included the method of exposure to BVDV by a PI calf, the procurement methods of the non-PI calves, and the broad distribution of individual BVDV antibody titers at arrival parallels typical scenarios encountered in stocker,

back-grounding, and feedlot production systems throughout the U.S.<sup>29-32,36</sup> These attributes, coupled with the ability to measure natural BVDV transmission among PI and non-PI cattle by *rRT-PCR* analysis and confirmed by seroconversion, indicates that this model for evaluating BVDV transmission from PI to non-PI individuals is valid, reproducible, and offers advantages for evaluating natural BVDV transmission by co-mingling individuals.

Diagnostic sensitivity of VI performed on buffy coat to diagnosis BVDV TI has previously been observed to be superior to that of serum.<sup>27,28</sup> However, logistical concerns likely preclude private practitioners from collecting and submitting buffy coat. Anecdotal reports (as well as our own clinical experience) suggest that serum provides the veterinarian with a more convenient and practical sample to collect and process (compared to buffy coat) thereby being the more likely specimen to be submitted to diagnostic laboratories. Therefore, characterization of TI cattle by means of serum (not buffy coat) was determined to be the primary sample in the present study.

These data suggest that cattle exposed to a PI BVDV individual can rapidly become TI with BVDV (as assessed by *rRT-PCR* on serum samples and indicated by a four-fold rise in BVDV antibody titers to the homologous PI strain) while lacking positive VI status in serum and discernible clinical disease. These findings are complemented by positive *rRT-PCR* outcomes on nasal swabs denoting that BVDV nucleic acid was circulating in the population thereby serving as a potential source for further transmission to co-mingled cohorts. Furthermore, the positive VI on buffy coat samples from selected TI calves matched the BVDV sequenced from the respective PI calves indicating that nucleic acid identified by *rRT-PCR* did originate from infectious virus shed by the PI calf in each study. To our knowledge, this is the first report characterizing this form of TI of BVDV by natural exposure by co-mingling of non-PI individuals with PI individuals. Previous studies depicting transient infections from natural BVDV exposure have isolated BVDV in serum and buffy coat by VI methods.<sup>25-28</sup> However, our findings of a four-fold rise in serum antibody titers to the homologous BVDV strain shed by the PI calf (experienced by a majority of the non-PI sample population) coupled with positive *rRT-PCR* results among serum samples and nasal swabs is clearly indicative of BVDV circulating within in each group of non-PI calves.

Despite the lack of positive serum VI findings among non-PI cattle in the present study, *rRT-PCR* results indicate that BVDV nucleic acid can be identified by *rRT-PCR* in serum from

days 6 – 21 post-PI exposure with subsequent duration of positive *r*RT-PCR results for approximately 3 – 4 days. Brownlie et al. (1987) described that BVDV antibody-negative non-PI cattle were viremic from days 4 – 7 post-BVDV.<sup>22</sup> Unfortunately, this report does not provide the methodology of how BVDV exposure occurred (experimental or natural exposure) or how viremia was diagnosed (compared to antibody production and *r*RT-PCR findings in the present study); nonetheless, it does demonstrate a similar duration of TI (~ 3 – 4 days) with a tighter timeframe of TI onset than what was observed in the present study. Conversely, previous authors have observed that BVDV could be isolated from the buffy coat of antibody diverse groups of non-PI calves from days 6 – 35 when exposed to PI cattle.<sup>27,28</sup> This suggests that an immunologically diverse population of cattle (as in the present study) may display a high degree of variability in the timing of onset of BVDV. This could be attributed to the pathogenicity of the virus shed by the PI animal, overall herd immunity, or by behavioral dynamics (i.e. differences in the rate of intermingling) that differ among populations.

One potential variable leading to an inability to isolate BVDV by VI in serum among TI cattle may be attributed to the characteristics of BVDV shed by the PI calves. The PI calves in both studies were infected with BVDV Type 1b; previously shown to be the type of highest prevalence in U.S. feedlots.<sup>37,38</sup> These PI calves possessed quantifiable serum BVDV concentrations and positive nasal swab samples at multiple sample times indicating that they were both truly PI and currently shedding BVDV. However, the quantity of BVDV in serum among the PI calves in the present study was relatively less ( $10^2 - 10^3$  CCID-50/mL) when compared to previously reported serum BVDV concentration of PI cattle.<sup>3,25,26</sup> Although a recent study proposed that BVDV concentrations in nasal swabs may be superior to that in serum or buffy coat for predicting the viral load shed into the environment,<sup>10</sup> serum concentrations from the PI calves in the present study may indicate that low concentrations of BVDV were shed by PI cattle.

In addition to the reduction in viral shedding by the PI calves (as described above), the lack of clinical disease in the present study may be partly explained by the antibody diversity of the study population. Previous researchers have described variable results when attempting to measure the association between BVDV antibody status and the risk of BVDV infection. A previous study failed to detect a significant association among VI outcomes and BVDV antibody status among non-PI calves.<sup>27</sup> Conversely, antibody diverse non-PI cattle vaccinated with a

commercial BVDV vaccine were observed to have a reduced risk of becoming VI positive on buffy coat samples when compared to unvaccinated calves upon exposure to PI cattle.<sup>28</sup> Further research in externally valid production environments is needed to quantify the effects of prior BVDV antibody production, either by natural infection or by vaccination, on incidence and duration of subsequent viremia and clinical disease following exposure to PI cattle shedding different genotypes and variable concentrations of BVDV.

The nasal swab findings from the present study concur with prior research which observed a lack of BVDV detection (by VI) in nasal swab samples among non-PI cattle when exposed to PI calves.<sup>25,27,28</sup> However, a previous study observed the ability of VI to identify BVDV shedding in nasal secretions among antibody-negative TI cattle upon prolonged exposure to a PI calf.<sup>26</sup> Although the ability of TI cattle to infect non-PI cohorts was not measured in the present study, positive *rRT-PCR* results on nasal swab samples from the non-PI population (with variable levels of BVDV antibodies to the homologous PI strain) suggest that BVDV (or at least viral nucleic acid) was being shed in nasal secretions potentially affording TI cattle the capability of transmitting the virus. Previous studies have observed that TI cattle can potentially transmit BVDV to susceptible populations over long durations with and without the presence of PI cattle.<sup>26,39,40</sup> These data suggest that BVDV transmission may not only be associated with virus excreted from PI cattle but that TI cattle may contribute to viral transmission. Nonetheless, further research is warranted to determine the role that TI cattle play in the transmission of BVDV within production systems.

Prior research has associated PI cattle with clinical disease and performance loss in both the cow-calf and feedlot sectors of the beef industry.<sup>41-44</sup> However, other authors have demonstrated that the presence of PI cattle did not pose a negative health or performance threat to non-PI cattle in the same pen.<sup>36-39</sup> Prior reports, as well as the present study, demonstrate the potential variation in health and performance outcomes that may be experienced by cattle populations when PI cattle are present. Despite the lack of clinical disease in this study, the high percentage of cattle becoming TI indicates that subclinical BVDV infections may be more prevalent than previously described.<sup>45</sup> Unfortunately, we were unable to capture individual weights of calves in either study to determine average daily gain and the potential association of transient BVDV infections with performance loss despite a lack of clinical disease. Therefore,

further research will be needed to determine the prevalence of subclinical, transient BVDV infections and the potential impacts on feed performance and carcass characteristics.

The disease transmission model used in the present study was twice observed to be an effective means of studying viral transmission characteristics of BVDV among PI cattle and immunologically heterogeneous populations of non-PI cattle. This model simulates current industry management practices thereby providing a relatively high degree of external validity to the conclusions of the research. The findings indicate that when commingled non-PI cattle are exposed to a PI individual, they can become TI to BVDV based on *r*RT-PCR and seroconversion (in our study indicated by a four-fold rise in  $\alpha$ -BVDV antibodies to the homologous BVDV strain recovered from the PI individual). Results of BVDV *r*RT-PCR indicates that BVDV nucleic acid can be identified in the serum of co-mingled cattle beginning approximately 6 days post-exposure to the PI calf and remaining for approximately 3-4 days in duration. These findings were observed despite a lack of positive serum VI findings or clinical disease. The lack of both observable clinical disease and inability to isolate BVDV may have been attributed to the variability in preexisting antibody titers to BVDV among the non-PI calves and unknown strain/virulence characteristics of the BVDV being shed by the PI calves used in this study. Our data indicates that VI performed on serum and nasal swabs may lack the sensitivity for diagnosing many transient BVDV infections. Additionally, our results indicate that BVDV transmission from PI individuals can occur rapidly to co-mingled individuals without overt clinical disease that might be an indicator of exposure. These results also indicate that we as veterinary professionals might need to reassess our timetable for testing to define PI BVDV individuals if the rationale for PI testing is to limit BVDV transmission to a naive or susceptible population. Current testing is often completed after extensive co-mingling of animals of unknown status in stocker, back-grounding, and feedlot production systems. These results would indicate that exposure to PI individuals in these scenarios might be too late to avoid the consequences of BVDV transmission and infection on other co-mingled individuals. This approach might indicate and justify earlier PI-BVDV testing (i.e. at the cow-calf production level).

Despite a lack of positive VI findings on serum and nasal swab samples among the non-PI sample population, numerous positive serum and nasal *r*RT-PCR results (and limited VI on buffy coat samples) could be generated from these samples and seroconversions of all non-PI

individuals evaluated in these studies, strongly supports our conclusions that BVDV infections with accompanying viral shedding occurred among non-PI cattle in this study due to co-mingling with a PI individual. Cattle TI with BVDV may contribute to enhanced BVDV transmission to additional co-mingled animals under these conditions; however this was not evaluated in the current study.

## Sources and Manufacturers

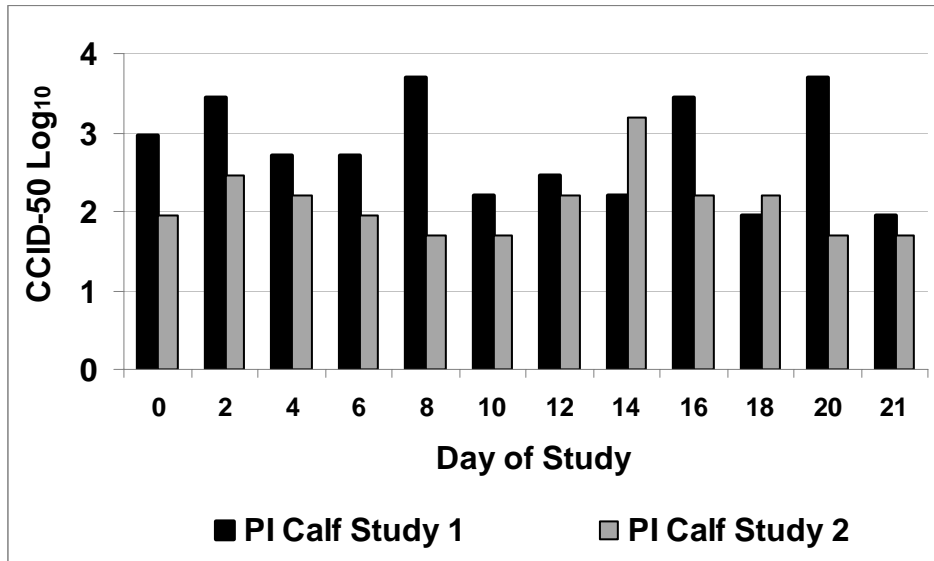
- a. Vision 7<sup>®</sup>, Intervet/Schering Plough Animal Health, The Netherlands
- b. Dectomax<sup>®</sup>, Pfizer Animal Health, Kalamzoo, MI
- c. Prozap<sup>®</sup> Insectrin<sup>®</sup> Pour-On XTRA, Chem-Tech, LTD., Des Moines, IA
- d. Large Ear Notcher, Stone Manufacturing, Kansas City, MO
- e. BD Universal Viral Transport, cat.# 220221, Becton, Dickinson & Co., Sparks, MD, 21152
- f. Draxxin<sup>®</sup>, Pfizer Animal Health, Kalamzoo, MI
- g. Nufor<sup>®</sup>, Intervet/Schering Plough Animal Health, The Netherlands
- h. Biomycin 200<sup>®</sup>, Boehringer Ingelheim, Saint Joseph, MO
- i. Fluorescen (FITC) conjugated Affini Pure Goat Anti-Mouse IgG (H & L), code # 115-095-003, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA
- j. MagMax<sup>™</sup>-96 Viral RNA Isolation kit, Ambion, Austin, TX
- k. MagMax Express<sup>®</sup> magnetic particle processor, Applied Biosystems, Austin, TX
- l. Magmax Express 96 protocol AM1836\_dw\_100V2, Applied Biosystems, Austin, TX
- m. BVD RNA Test kit, Applied Biosystems, Austin, TX
- n. 25X BVDV Control RNA and Xeno<sup>™</sup> RNA control, Applied Biosystems, Austin, TX
- o. 7500 Fast Real-Time System, Applied Biosystems, Austin, TX
- p. 7500 SDS (v1.4) software, Applied Biosystems, Austin, TX
- q. Microsoft Excel<sup>®</sup>, 2003, Redmond, WA
- r. Proc Univariate, SAS (version 9.1), Cary, NC

**Table 4.1: Individual animal outcomes among the non-persistently infected calf population across studies one (n=12) and two (n=15). Serum and nasal swabs were collected every other day and every fourth day (including day 21), respectively. Cumulative serum and nasal swab PCR status reflects the individual animal status at day 21 (post PI introduction).**

<b>Calf</b>	<b>Study</b>	<b>Antibody titer to homologous PI BVDV strain at arrival</b>	<b>Cumulative Serum PCR status</b>	<b>Day of positive PCR Onset</b>	<b>Duration of positive PCR status (days)</b>	<b>Cumulative Nasal Swab PCR status</b>
1	1	0	+	8	2	+
2	1	0	+	10	3	-
3	1	0	+	8	7	-
4	1	8	+	10	2	+
5	1	8	+	10	3	-
6	1	16	+	8	2	+
7	1	16	+	16	2	+
8	1	16	+	6	3	-
9	1	16	-	Not available	Not available	+
10	1	64	+	6	5	+
11	1	64	-	Not available	Not available	+
12	1	128	+	6	3	+
1	2	0	+	14	2	+
2	2	0	+	12	3	+
3	2	0	+	10	3	+
4	2	0	+	6	3	+
5	2	0	+	8	3	+
6	2	0	+	8	7	+
7	2	0	+	8	9	-
8	2	0	+	10	9	+
9	2	0	+	10	9	+
10	2	8	+	14	2	-
11	2	8	-	Not available	Not available	+
12	2	8	-	Not available	Not available	+
13	2	16	+	12	2	-
14	2	32	+	21	1	+
15	2	256	-	Not available	Not available	+



**Figure 4.1: Individual serum BVDV concentrations for the PI calves in studies one and two at each sample collection time point across a 21 day study period. The cell-culture infectious dose<sub>50</sub>/ml (CCID-50) values (y-axis) reflect serum viral concentration in log<sub>10</sub> format (e.g. log<sub>10</sub> 3 = 10<sup>3</sup>).**



# **CHAPTER 5 - Bovine Viral Diarrhea Virus (BVDV)**

## **Status in cow-calf herds, its association with biosecurity practices, and the association of these practices with self-perceived understanding of BVDV among Montana beef producers**

### **Introduction**

Bovine viral diarrhea virus (BVDV) has long been recognized as a pathogen with the ability to manifest in a variety of clinical syndromes.<sup>139,153,230,231</sup> The viral reservoir, cattle persistently infected (PI) with the virus, are created by *in utero* infection occurring during days 45-125 of gestation and are thereby lifelong shedders of BVDV.<sup>56</sup> Therefore, the creation of PI cattle in both beef and dairy production systems can be attributed to the maternal segments of each respective industry.

Numerous tools have been implemented to minimize production losses attributed to PI cattle among herd and pen mates. Currently, several testing modalities are commercially available for identification of individual PI cattle.<sup>83,145,146,148-150,153</sup> Additionally, vaccination programs are considered an important component of most BVDV control programs within the United States and are intended to elevate herd immunity and reduce the risk of fetal infection.<sup>159,161,176,232</sup> Prevention of BVDV introduction into a beef herd is also an important attribute of BVDV control programs. Biosecurity has previously been defined as the efforts to prevent the introduction of pathogens or toxins that have the potential to damage the health of a herd of cattle or the safety of a food product.<sup>157</sup> Therefore, biosecurity programs focus on minimizing the risk of pathogen exposure by implementing specific cattle management and procurement practices.<sup>155</sup>

The Montana BVD-PI Herd Biosecurity Project was created in 2006 as a segment of the Montana Beef Quality Assurance program. The overarching goal of this program is to reduce the risk of introducing BVDV into Montana beef herds by providing cow-calf producers with BVDV education, BVDV herd risk assessment, testing protocols to screen herds for PI cattle, and strategies to optimize herd biosecurity and management.<sup>233</sup> The data compiled from this project provides a unique opportunity to estimate the relationship among common management

practices and herd BVDV status. These findings can potentially be implemented into current BVDV preventative and control programs in order to modify and/or strengthen current recommendations.

The objective of this study was three-fold: 1) estimate the herd and within-herd prevalence of BVDV among program participants in Montana, 2) estimate the distribution of specific management practices of beef cow-calf participants during the three years prior to their individual involvement in the program and determine if significant associations are present among the management practices of cow-calf herds and herd BVDV status, 3) determine if associations are present between the individual producer's perceived knowledge of BVDV and the implementation of specific management practices.

## **Materials and Methods**

### ***Sample Population***

Implemented in 2006, the Montana BVD-PI Herd Biosecurity Project is a voluntary program open to all beef producers within the state of Montana. Upon herd enrollment, producers were provided with the necessary materials for sample collection based on the number of animals stated by the producer that would be tested for BVDV. Individual animal diagnosis was performed by a previously described technique<sup>149,150</sup> currently offered by a private commercial laboratory<sup>a</sup>. Individual animal results were communicated to both the producer and the project coordinators. Test results and management considerations were then discussed among the herd owner, the project coordinators, and the herd veterinarian to determine the optimal management protocol given the herd BVDV status.

The sample population in this study included all past and active participants of the Montana BVD-PI Herd Biosecurity Project since its inception in 2006 and extending through 2009. The authors were supplied with individual herd information consisting of herd size, classes of cattle tested for BVDV, and annual BVDV test results thereby providing the means to classify herds as either of BVDV positive or negative status and determine within-herd BVDV prevalence among participating herds. All herds were subsequently surveyed to evaluate objectives 2 and 3.

### ***Survey Instrument***

The survey consisted of six open and seven nominal scalar (closed) questions designed to capture information regarding various cow-calf herd management practices. The questions were designed to determine herd size (1 question), replacement female/male procurement and management practices (3 questions), BVDV vaccination implementation and timing of administration (2 questions), procurement and management of stocker cattle in relation to the resident herd (2 questions), communal grazing practices of the resident herd (2 questions), the practice of transporting cattle off-site followed by reintroduction to the resident herd (2 questions), and individual understanding of BVDV and its effect on production systems (1 question). To maintain anonymity but yet provide identification, each participant was randomly assigned a unique number, produced by a commercial software package<sup>b</sup>, and merged onto their respective survey. This survey was approved by the Kansas State University Committee for Research Involving Human Subjects Institutional Review Board. A copy of the 4-page survey may be obtained from the authors upon request.

### ***Survey implementation***

In order to contact the program participants, home addresses were obtained from the Montana State University Beef Quality Assurance program. Upon receipt of the survey, each respondent was requested to return the completed survey within 30 days of receipt. All surveys were returned to the co-author (CP; in Montana) and subsequently delivered to Kansas for data entering and analysis.

### ***Statistical analysis***

The experimental unit was the herd throughout all analyses and the case definition of a positive herd was a herd previously diagnosed with  $\geq 1$  PI animal (yes or no) during its tenure in the Montana BVD-PI program. Potential associations were evaluated between survey responses (independent variables) and both herd BVDV status and herd self assessment of BVDV knowledge (dependent variables). Data were entered into a commercial software package<sup>b</sup> and descriptive statistics were calculated. Independent categorical variables displaying sparse data within individual levels were collapsed with adjacent categories to provide sufficient numbers of observation among levels. Linearity between the remaining continuous independent variables and dependent variables was assessed by categorizing the continuous variable by quartiles or

natural breaks in the data and analyzed by commercial software<sup>c</sup>; continuous independent variables failing to demonstrate linearity remained as categorical variables. Evidence of correlation was assessed by correlation matrices (continuous responses) as well as contingency tables (categorical responses). Correlation of  $\geq 0.4$  (continuous variables) or a significant association among two (categorical) variables ( $P < 0.05$ ) were considered potentially collinear. Among all models, each independent variable was assessed for association with the dependent variable by bivariable models using a liberal p-value ( $\leq 0.25$ )<sup>d</sup>. A forward step-wise model building procedure was then implemented to determine a final multivariable model; an alpha level of 0.10 was used for inclusion. Potential confounders were analyzed by measuring the change in the coefficient of the covariate of interest; a numerical modification of the coefficient by  $\geq 20\%$  (when the potential confounder was added to the model) suggested that significant confounding was present. Confounding variables were retained in the model regardless of statistical significance. All biologically plausible two-way interactions were also assessed following determination of main effects.

## **Results**

### ***Descriptive BVDV outcomes among project participants***

From 2006 through 2009, 585 beef herds voluntarily enrolled in the Montana BVD-PI Herd Biosecurity Project. The number of herds joining the program (for the first time) on an annual basis was 49 (8.4%) in 2006, 411 (70.3%) in 2007, 92 (15.7%) in 2008, and 33 (5.6%) in 2009, respectively. These participating herds represent 5.2% of the beef herds in the state of Montana.<sup>234</sup> Among the herds participating in the program, 38 were determined to possess  $\geq 1$  PI animal; thus, a herd BVDV prevalence of 6.5% (95% Confidence Interval [95% CI], 4.5% – 8.5%). The within-herd prevalence among these 38 positive herds ranged from 0.12% – 20.0% with a median value of 0.75% (Figure 5.1). Although not statistically evaluated, clustering of PI cattle among individual herds was evident as 68.4% (n=26) of BVDV positive herds detected  $> 1$  PI animal at the time of testing.

### ***Survey outcomes in the present study***

Of the 585 herds enrolled in the program from 2006 – 2009, 563 had BVDV results and were subsequently contacted to participate in the survey. Of these, 230 herds completed and

returned the aforementioned survey (40.9% response). Respondents included 95.7% (n=220) BVDV test-negative herds and 4.3% (n=10, 95% CI; 1.7%, 7.0%) BVDV positive herds.

**Herd Size** – The distribution of herd size among the survey respondent population is displayed in Table 5.1. Upon analysis, this variable was categorized by quartiles.

**Replacement female/male procurement and management practices** – Producers were asked to provide the number of purchased heifers, yearling bulls, pregnant heifers, pregnant cows, as well as home-raised heifers and bulls (raised off-site) that were subsequently introduced to the resident herd (Table 5.1). Upon analysis, data were categorized into individual binary formats (yes or no) thereby indicating whether producers either did or did not engage in procuring the above classes of cattle (Table 5.2). Among the respondents to this question, 82.5% (n=198/240) stated that they annually imported  $\geq 1$  of the aforementioned classes of cattle to the resident herd.

Among producers indicating importation of cattle, 33.8% (n=86/198) stated that they annually introduced pregnant females (heifers and/or cows) to the herd. Among these herds that import pregnant cattle, 91.9% (n=79) disclosed the method of procurement of pregnant heifers and/or cows. Of these individuals, 19.0% (n=15), 57.0% (n=45), and 24.1% (n=19) indicated they procured pregnant females outside of the resident herd by means of auction markets, private treaty, or both methods, respectively.

Respondents who imported cattle were asked to state the length of time (if any) that each of the above classes of cattle (excluding home-raised heifers and bulls) were quarantined from the resident herd prior to introduction (Table 5.1). Given the low number of responses, the distributions of the given data, and previous research estimating the length of transient infection of BVDV,<sup>63,66,67</sup> the above data was subsequently categorized in a binary format and analyzed to have (or have not) quarantined cattle  $> 14$  days from the resident herd prior to introduction. Open heifers, yearling bulls, pregnant heifers, and pregnant cows were isolated for greater than 14 days prior to introduction to the resident herd in only 31.9% (n=7/22), 39.5% (n=51/129), 35.6% (n=16/45), and 36.4% (n=16/45) of respondent herds, respectively.

**BVDV vaccination and timing of administration** – Among the respondents, 88.3% (n=190/215), 85.1% (n=86/101), and 76.9% (n=90/117) indicated that BVDV vaccine was annually administered to the cattle within the resident herd, home-raised replacement heifers/bulls (raised off-site), and leased/purchased replacement cattle, respectively, during the

three years prior to enrollment in the program. Among the entire respondent population (n=230), 49.2% (n=91), 30.8% (n=57), and 20.0% (n=37) indicated that BVDV vaccine was administered prior to breeding, at pregnancy diagnosis, or at both timepoints, respectively.

**Procurement and management of stocker cattle** – Among those that responded to this question (n=220), 10% (n=22) indicated that they had purchased stocker cattle during this timeframe. Among these 22 herds who purchased stocker cattle, 72.7% (n=16) indicated that the stocker cattle shared a pasture or had fence-line contact with the resident herd at some time point from breeding to weaning (i.e. were in contact with pregnant cattle).

**Communal grazing practices** – Descriptive statistics for respondents who allowed fence-line access and pasture-sharing among neighboring herds and the resident herd anytime during the breeding season through the weaning period are displayed in Table 5.1. Due to the low number of respondents indicating that pasture sharing did occur, no further analysis was performed on these data. Among respondents who indicated that fence-line access to neighboring herds did occur, data were categorized based upon fence-line exposure to 0-2, 3, 4, > 4 herds. Among respondents in the first (n=84), second (n=55), third (n=30), and fourth (n=53) categories, 15.6% (n=13), 22.8% (n=13), 35.3% (n=11), and 37.3% (n=20) indicated, respectively, that exposure to pregnant cattle within the resident herd occurred due to fence-line contact with neighboring herds.

**Transporting cattle off-site and reintroduction to the resident herd** – The number of respondents to this question regarding the various classes of cattle allowed to leave the premise and subsequently return to the resident herd is located in Table 5.2.

**Participant understanding of BVDV** – Among the respondents to a question regarding their understanding of BVDV (n=217), 73.0% (n=159) perceived themselves to be “fairly knowledgeable” in regard to BVDV, 26.1% (n=57) “knew some basics”, and 0.9% (n=1) “recognized the name, BVDV”. Due to the small number of responses in categories three, this last level was not included in further analyses.

### ***Association of survey outcomes and herd BVDV status***

Upon multi-variate analysis, herd BVDV status was associated ( $P < 0.10$ ) with the practice of annually vaccinating home-grown heifers and bulls (raised off-site) that are subsequently introduced to the resident herd (odds ratio [OR]=0.155, 95% confidence interval [CI]; 0.02-1.2).

Therefore, herds that annually vaccinated this class of cattle prior to introduction to the resident herd displayed approximately an 85% reduction in the risk of being BVDV positive. Although herd size was not significant, respondent herds possessing  $\geq 1000$  cows ( $n=12$ ) harbored 25% ( $n=3$ ) of the BVDV positive herds participating in the survey. All remaining survey outcomes were not significantly associated ( $P>0.10$ ) with herd BVDV status in the final model.

### ***Association of perceived understanding of BVDV and herd biosecurity practices***

A multi-variate model consisting of all herd management practices was then analyzed with regard to perceived knowledge of BVDV (i.e. producers that perceived themselves as “fairly knowledgeable” or “know some basics” about BVDV). Only covariates included in the model are discussed; therefore, reported data reflect model-adjusted estimates.

In this model, herd size was significantly associated ( $P<0.10$ ) with producer BVDV knowledge. Upon further analysis, this difference was observed to be driven predominately when comparing the fourth quartile (herd with  $> 375$  cows) to the bottom three quartiles. These findings suggest that producers of herd sizes  $> 375$  cows were significantly more likely ( $P<0.10$ ) to perceive themselves as “fairly knowledgeable” with regard to BVDV (OR=1.9; 95% CI, 1.1 – 3.2) when compared to all other categories.

Annual BVDV vaccine administered to the resident herd was significantly associated ( $P<0.10$ ) with perceived knowledge of BVDV (OR=2.7; 95% CI, 1.2-6.2). Therefore, herd owners that annually vaccinate the resident herd for BVDV are 2.7 times more likely to perceive themselves as “fairly knowledgeable” in regard to BVDV.

Communal grazing practices from breeding to weaning were significantly associated ( $P<0.10$ ) with perceived knowledge of BVDV (OR=0.45; 95% CI, 0.21 – 0.98). Therefore, program participants who engage in communal grazing are 55% less likely to perceive themselves as “fairly knowledgeable” with regard to BVDV.

The practice of transporting pregnant heifers off-site and subsequently reintroducing them to the resident herd was also significantly associated ( $P<0.10$ ) with being “fairly knowledgeable” with regard to BVDV (OR=14.7; 95% CI, 1.8 – 118.6). This suggests that producers who engage in this activity are 14.7 times more likely to perceive themselves as being relatively educated with regard to BVDV.

## **Discussion**



Herds in the Montana BVD-PI Herd Biosecurity Program had a BVDV herd prevalence of 6.5%. This finding is similar to previous studies that have determined herd BVDV prevalence among U.S. beef herds as well as those in western Canada.<sup>3,8,154</sup> However, to our knowledge, this is the first report providing herd prevalence data specifically for the state of Montana. Therefore, these findings from the present study suggest that only a small percentage of beef herds in the state of Montana are BVDV positive. However, given the distribution of within-herd PI prevalence, approximately 65% of BVDV positive herds possess > 1 PI animal; therefore, PI cattle tend to be clustered in a small percentage of herds as outlined elsewhere.<sup>3</sup>

Although the findings from this study highlight specific management practices implemented by participating herds, BVDV herd status was not shown to be associated with management practices previously recognized as risk factors for introducing BVDV to the resident herd. Given that the BVDV herd prevalence in the present study (6.5%) is low, these outcomes may be attributed to loss of statistical power thereby elevating the risk of type two errors.

Despite the lack of statistical associations of herd practices with BVDV status, the findings from the present study suggest that a large proportion of the participating herds engage in management practices previously described as having the potential to elevate the risk of disease introduction.<sup>157,158,235</sup> Smith et al 2009 reported that importation of pregnant beef heifers was a significant risk factor regarding the introduction of BVDV to resident herds when evaluated by a stochastic model.<sup>155</sup> In the present study, approximately 34% of respondents indicated that pregnant females had been previously brought into the herd within three years of enrolling in the program. Although importing cattle into the resident herd increases the risk of disease introduction, implementation of quarantine procedures may help reduce the probability of this occurrence by allowing time for transient infections to wane, vaccines to be administered and take effect, and for diagnostic tests to be performed. In addition to exposing the resident herd to outside sources of cattle, 25.5% of respondent herds in the present study indicated that they participated in communal grazing activities. This estimate closely parallels survey data in a previous report regarding western U.S. beef producer's participation in some variety of communal grazing (24%).<sup>157</sup> This practice is common among states in the western U.S. and is necessary for providing adequate levels of forage to cow herds during the grazing months. Therefore, communal grazing is not likely to be curtailed solely due to its effect on the risk of

BVDV introduction. However, the obvious hazard of communal grazing is attributed to the commingling among multiple herds thereby increasing the risk of disease transmission. Instituting biosecurity measures such as herd testing protocols for BVDV (prior to commingling) and quarantine protocols prior to reintroduction may reduce the risk of introducing economically devastating disease syndromes to the resident herd.

In the present study, annually administering BVDV vaccine to home-grown heifers and bulls prior to introduction to the resident herd significantly reduced the risk of a positive herd BVDV status. In heifers, the introduction of BVDV to the herd may be attributed to the production of PI calves. Pre-breeding administration of BVDV vaccine to heifers should not be viewed as the sole element of BVDV prevention; however, prior research has displayed significant reduction in fetal infection and subsequent PI calf production among vaccinated heifers when compared to unvaccinated controls.<sup>113,159,161,173,175,176,236</sup>

In the present study, owners of larger herds (> 375 cows) were significantly more likely to perceive themselves as “fairly knowledgeable” with regard to BVDV. Potential reasons for this finding could be that the cow-calf enterprise of larger producers is their primary source of income; therefore, these individuals may be more likely to be familiar with BVDV due to greater exposure to their herd veterinarian and a greater interest in the beef industry (compared to smaller producers). Alternatively, simply due to sheer numbers, larger herds may be more likely to have previously experienced actual clinical effects associated with BVDV than smaller herds, subsequently forcing them to be highly aware of BVDV. In addition to herd size, the data in the present study suggest that producers who understand BVDV may avoid certain management practices that potentially increase the risk of disease introduction. Beef producers in the present study who considered themselves “fairly knowledgeable” in regard to BVDV were more likely to institute annual BVDV vaccine programs within the resident herd and avoid communal grazing during the timeframe of breeding to weaning. This suggests that prior BVDV education (coming from numerous potential sources such as first-hand experience with BVDV, experiences of other producers, veterinarian consultation, as well as state and national organizations) has improved client understanding and compliance with BVDV control protocols. These findings contradict a previous study that observed a poor association between producer knowledge and compliance with BVDV control programs.<sup>237</sup>

Conversely, in the present study, producers indicating a high level of BVDV knowledge were significantly more likely to transport pregnant heifers off-site while subsequently reintroducing them to the resident herd at a later time. Without proper precaution, this practice may be hazardous due to the unknown PI status of the unborn fetus upon return. These findings suggest that either beef producers don't recognize this practice as being a potential biosecurity risk or other constraints such as pasture management, breeding/calving supervision, labor allocation, or forage availability forces herds to adopt this practice in order to optimize herd production. One preventative measure aimed to potentially reduce the risk of BVDV introduction by this practice would be segregate pregnant heifers upon return to the resident herd until both the heifer and calf are confirmed free of BVDV. This final variable signifies that continued education is needed at the herd level in order to reduce the risk of BVDV introduction.

Despite the present findings, sources of bias were likely present in this study. Non-response bias may have been introduced as herds previously diagnosed as being BVDV positive may have been less likely to participate in the present study. Analysis of the non-response among BVDV positive herds (73.9%, 95% CI; 59.0%, 88.4%) compared to the non-response among BVDV negative herds (59.6%, 95% CI; 55.5%, 63.7%) indicates that these two proportions are not significantly different. Additionally, further analyses comparing the aforementioned herd prevalence estimate for the respondent population (4.3%; 95% CI, 1.7% – 7.0%) and that of the non-respondent population (8.4%; 95% CI, 5.4% – 11.4%) indicate that the proportion of BVDV positive herds among respondents and non-respondents were not statistically different. This evidence suggests that non-response bias did not significantly alter the estimates in the current study. Recall bias may have impacted this study as participants were asked if certain management practices were being utilized three years prior to volunteering in the Montana BVD-PI program. Therefore, given the year this program was founded (2006), it is likely that some participants may be unable to specifically remember how long a management practice has been employed or when it was begun thereby potentially affecting the accuracy of individual responses. Survey respondents did not consist of a random sample as the study population consisted of herds that voluntarily participated in the Montana BVD-PI program. Nonetheless, geographical distribution of BVDV positive herds (data not shown) displayed a relatively uniform distribution throughout the state of Montana; therefore, it is likely that the survey responses of positive herds in the present study would be typical of other positive herds.

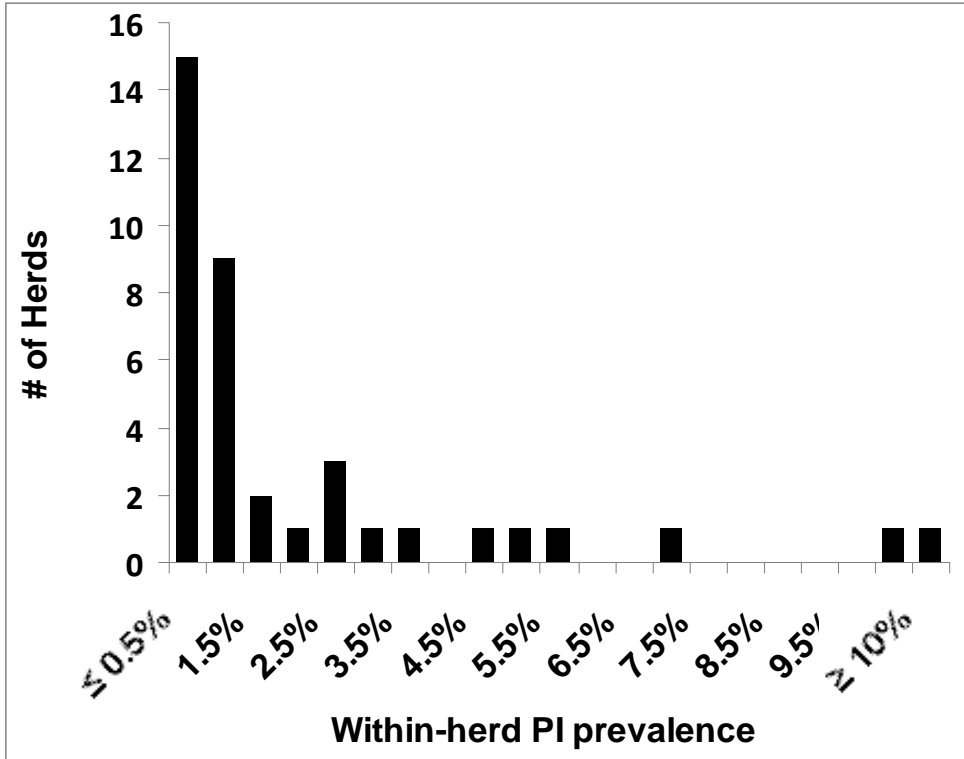
Likewise, herd BVDV status was determined *a priori* of the present study. However, closer inspection of diagnostic records indicated that a small number of herds failed to test the calf population and tested only replacement cattle or the mature cows (i.e. failing to test the subpopulation [i.e. calves] at greatest risk of housing PI cattle). Therefore, the present herd and within-herd prevalence estimates may be slightly underestimated. Lastly, due to the cross-sectional nature of this study, we are unable to determine if reverse causation was present due to the inability of determining if the modeled fixed effects (e.g. BVDV vaccination of home-raised replacement heifers) occurred before or after the modeled response variables (e.g. herd BVDV status).

In summary, the present data suggests that a small percentage of beef herds in Montana are infected with BVDV. However, among those positive herds, the within-herd distribution of PI cattle is large thereby suggesting that PI animals are indeed clustered at the level of the farm. These data also provide evidence that many beef producers in Montana currently engage in management practices that may elevate the risk of eventual disease introduction. Veterinarians and beef producers must constantly assess their production practices and determine if a biosecurity risk is present. However, instituting any biosecurity program must be determined to be of economic benefit to the producer based upon the prevalence of the disease and the individual producer's risk aversion to the pathogen. Continued persistence among veterinarians, academic institutions, extension services, and state and national organizations is necessary to maintain and improve producer education to optimize BVDV control and prevention.

## **Sources and Manufacturers**

- a. Animal Profiling International, Inc., Portland, OR
- b. Microsoft Excel<sup>®</sup>, 2007, Redmond, WA
- c. Proc GLM, SAS (version 9.1), Cary, NC
- d. Proc Logistic, SAS (version 9.1), Cary, NC

**Figure 5.1: Within-herd prevalence of PI cattle among all BVDV positive herds (n=38) voluntarily enrolled in the Montana BVD-PI Biosecurity Program from 2006 – 2009.**



**Table 5.1: Descriptive statistics of continuous variables among the respondent population (n=230) that provided answers to the following questions.**

Survey Question	# of Respondent herds	Mean	Median	S.D.*	Minimum Observation	Maximum Observation
<b>Herd Size</b>	229	323	200	399	3	3000
<b>Cattle (purchased or home-grown) raised off-site and introduced to resident herd</b>						
heifers	213	6.4	0	40.3	0	500
yearling bulls	223	3.0	2	4.8	0	40
pregnant heifers	215	6.0	0	25.8	0	300
pregnant cows	211	8.0	0	30.0	0	250
home-raised heifers and bulls	212	30.0	0	60.0	0	375
<b>Number of days that purchased cattle are quarantined prior to introduction to resident herd</b>						
heifers	22	19.0	10	27.0	0	90
yearling bulls	129	19.0	7	27.0	0	180
pregnant heifers	45	16.0	10	20.0	0	90
pregnant cows	44	12.0	10	14.0	0	60
<b>Number of neighboring herds allowed fenceline contact with resident herd (breeding to weaning)</b>	222	3.5	3	2.5	0	15
<b>Number of neighboring herds that shared pasture with resident herd (breeding to weaning)</b>	225	0.6	0	1.5	0	10
<b>Number of days that home-raised cattle, transported off-site, are quarantined prior to introduction to resident herd</b>						
Heifers (breeding)	27	39.4	0	79.3	0	365
Heifers (exhibition)	14	2.5	0	5.2	0	14
Pregnant Heifers	28	13.0	0	38.3	0	180
Pregnant Cows	45	8.2	0	24.6	0	125
Bulls	55	13.9	0	41.8	0	250

**Table 5.2: Descriptive statistics of categorical variables among the respondent population (n=230) that provided answers to the following survey questions.**

Survey Question	Number of Respondents (herds)	Did the herd engage in the management practice?	
		Yes (%)	No (%)
<b>Replacement cattle leased, purchased, or raised off-site and introduced to resident herd</b>			
heifers	213	11.3%	88.7%
yearling bulls	223	66.1%	33.9%
pregnant heifers	215	21.3%	78.7%
pregnant cows	211	19.1%	80.9%
home-raised heifers and bulls	212	43.0%	57.0%
<b>Transporting cattle off-site with subsequent reintroduction to resident herd</b>			
heifers (for breeding)	218	10.6%	89.4%
heifers (for exhibition)	214	5.6%	94.4%
pregnant heifers	219	11.0%	89.0%
pregnant cows	221	15.4%	84.6%
bulls	222	19.4%	80.6%



# CHAPTER 6 - Quantification of the Value of Implementing Whole Herd Testing Strategies in U.S. Beef Herds

## Introduction

Bovine viral diarrhea virus (BVDV) is an endemic disease of the U.S. beef herd with the potential to clinically manifest by numerous pathways. The virus has been observed to adversely affect cow-calf herds by inflicting reproductive wastage,<sup>1-4</sup> elevate the incidence of morbidity and mortality,<sup>1-4</sup> and reduce weaning weight.<sup>1</sup> One unique aspect of BVDV is its carrier state which consists of cattle persistently infected (PI) with the virus. Cattle PI with BVDV are created by *in utero* viral exposure during days 42 – 125 of gestation.<sup>5</sup> Upon parturition, these animals shed the virus in all bodily secretions and excretions thereby increasing the risk of exposing non-PI cattle to BVDV.<sup>5-8</sup> Therefore, one primary goal of BVDV control programs is to identify and eliminate PI animals from the herd.<sup>9-12</sup>

Given the negative effects associated with BVDV and the economic ramifications associated with its presence, numerous testing modalities have been developed in order to accurately identify PI cattle. These testing methods include immunohistochemistry (IHC) on formalized skin tissue,<sup>13-18</sup> antigen-capture ELISA (ACE) on the phosphate-buffered saline (PBS) fraction of PBS soaked skin tissue,<sup>15,16,19-21</sup> and real-time polymerase chain reaction (PCR) performed on fresh skin tissue.<sup>22</sup> Although these tests are relatively accurate at discerning among PI and non-PI cattle, very little data exists for comparison of test strategies (i.e. one single test versus two-test protocols interpreted in series).

Current recommendations suggest that cow-calf herds at high risk of being positive for BVDV should institute a whole herd testing protocol in order to identify and eliminate all PI cattle.<sup>9,11</sup> Despite a number of reports in the literature that associates BVDV with health and production loss,<sup>1-4,23-25</sup> a moderate body of literature reports no adverse effects among cattle exposed to PI animals in both the cow-calf and feedlot sectors of the beef industry.<sup>26-28</sup> Additionally, the herd prevalence of PI cattle among cow-calf herd in the United States (U.S.) has previously been shown to be  $\leq 10\%$  indicating that the majority of beef herds do not possess

PI cattle.<sup>1,29,30</sup> Therefore, given the large variation in clinical outcomes upon exposure to PI cattle and very few herds within the U.S. are BVDV positive, the economic viability of whole-herd testing programs has been questioned.

Given that numerous European countries have instituted national BVDV eradication programs within the last two decades,<sup>31-34</sup> the economic justification for implementing a nationwide BVDV eradication campaign in the United States (U.S.) among different sectors of the beef industry has not been evaluated. In addition, herd size and herd prevalence (or the probability that a given herd is BVDV positive) may impact the value of whole herd testing protocols. Lastly, due to inherent variation in the cost and accuracy of currently offered tests, the value of individual BVDV testing strategies (i.e. one time tests and two-test combinations) may be impacted by herd size and herd prevalence. The objective of this study was to utilize Monte Carlo simulation to determine the relative value of various whole-herd BVDV testing strategies among different herd sizes and herd prevalences within beef cow-calf herds in the U.S.

## **Materials and Methods**

### ***Model structure***

A spreadsheet model was constructed to estimate the value of implementing various whole-herd BVDV testing strategies in cow-calf herds across a two year timeframe in a spring calving beef herd. A Monte Carlo model was initially constructed to estimate the gross income in a cow-calf herd based on probability distributions of known performance variables and BVDV herd status. Within each iteration of the model, gross income from the cow-calf herd was estimated over a two year period when either applying or not applying the testing strategy. The outcome variable was the difference in income between either applying or not applying the testing strategy. Potential associations with the outcome variable were evaluated with testing strategy, herd size, and herd prevalence.

The value of testing for PI cattle was estimated by incorporating three commonly performed BVDV tests (immunohistochemistry [IHC], antigen-capture ELISA [ACE], and real-time reverse transcriptase polymerase chain reaction [PCR]) into 12 different testing strategies analyzed as either a single test (3) or as two-test strategies (9) applied sequentially and interpreted in series (Table 6-1). Three different herd sizes (50, 100, and 500 breeding females) were to represent the range in cow-calf operational sizes within the U.S.

Each BVDV testing protocol was applied at three herd prevalence values (7.7%, 19%, 47%) selected from previous literature<sup>1,29,30</sup> to represent a range of realistic herd prevalence. Likewise, these values may also represent the probability (0.077, 0.19, and 0.47) that any given herd is BVDV positive. Separate simulations were performed for each testing strategy and herd prevalence permutation in each respective herd size.

Baseline distributions (i.e. non-BVDV associated) for health and performance parameters consisting of preweaning morbidity,<sup>35</sup> health costs of morbid cattle,<sup>36-38</sup> preweaning mortality,<sup>35,39-44</sup> weaning weight,<sup>45</sup> and cull cow %<sup>46</sup> were determined from available literature (Table 6-2). The same modeled estimates for health and production were used in both years of the model to ensure that these two years were not independent of one another but were correlated as would be expected in a herd from one year to the next. For example, given the distribution of weaning weights in Table 6-2 (and the random method sampling of Monte Carlo simulation), failing to correlate weaning weights in both years could presumably allow for vast (and therefore unlikely) differences in the average weaning weights from one year to the next regardless of BVDV prevalence or testing strategy.

Market prices for weaned cattle and cull cows were based on a 10 year average (1999 – 2009) for 249.5 kg (550 lb) steers (\$110/45.4 kg, [\$110/cwt]) and utility cow price (\$43/45.5 kg, [\$43/cwt]), respectively (Table 6-2).<sup>47</sup> For weaned cattle, a price slide of \$0.05/0.45 kg (\$0.05/lb) was implemented whereas all cull cows were assumed to be classified as utility grade. The bull:cow ratio was assumed to be 1 bull for every 20 females regardless of herd BVDV status.<sup>48</sup>

The within-herd BVDV prevalence (for both calves and mature animals)<sup>1,15,49-51</sup> was modeled based on estimates from prior data (Table 6-2). For each simulation, if the herd was negative for BVDV, the within-herd BVDV prevalence was forced to be 0% and the year one calving percent was determined from the baseline distribution (Table 6-2). Conversely, if the herd was deemed to be BVDV positive, the initial calving percent in year one (determined from the aforementioned distribution) was subsequently reduced due to PI cattle being in contact with pregnant cattle during the previous gestational period (Table 6-3).<sup>1-4</sup> Therefore, the overall calving percent in BVDV positive herds was calculated by subtracting the modeled reduction in calving attributed to BVDV from the modeled baseline calving percent. The number of open cows at the beginning of year one (for both BVDV positive and negative herds) was thereby

calculated by subtracting the number of live calves from the original herd size (i.e. 50, 100, or 500 breeding females). Income calculated based on the performance of this herd was used as the baseline for comparison to the estimated herd income after test strategy application.

After baseline calculations, the impact of applying the selected BVDV testing strategy to the herd was evaluated. In the present study, a whole-herd BVDV test was defined as testing all live calves, open cows and bulls, and cows from test-positive calves.<sup>9</sup> Therefore, the model assumes that BVDV tests were performed on calves shortly after birth (in the spring) to remove PI cattle early in life thereby avoiding further exposure to the cow herd during the subsequent breeding season and to the remaining calf crop during the subsequent months.

The present study evaluated 12 BVDV testing modalities that included both single and two-test protocols (Table 6-1). The cost of individual BVDV tests and test set-up fees were modeled as probability distributions according to prices offered by numerous veterinary diagnostic laboratories in the U.S. (Table 6-4). These fees reflect prices of individual tests as of January 1, 2010. In regards to diagnosing PI cattle in the present study, single test protocols defined a PI diagnosis based on a single positive test result. Conversely, testing modalities requiring two tests were applied in a sequential manner with series interpretation. Therefore, only cattle testing positive on the initial test were evaluated by the second test; consequently, a diagnosis of being a PI animal (in a multiple test protocol) was only confirmed after both tests were observed to be positive. Diagnostic sensitivity (Se) and specificity (Sp) estimates for IHC, ACE, and PCR were determined from distributions gleaned from available literature (Table 6-5).

In the field, performing diagnostic tests sequentially (and interpreting in series) is implemented in order to elevate diagnostic Sp thereby increasing the positive predictive value of the test (compared to a single test protocol). In two-test protocols, conditional independence presumes that the outcome of the second test is independent of the initial test (regardless of the outcome on the initial test). In reality, diagnostic tests that identify similar molecules (e.g. antibodies, antigen) are biologically related; therefore, when implementing a testing strategy that uses multiple tests to detect BVDV antigen, cattle that test positive on the initial test are more likely to test positive on the confirmatory test. This lack of test independence has been previously described as test covariance.<sup>52,53</sup> Therefore, despite the ability of serial interpretation to increase test Sp, the augmentation may not be as vast as expected when assuming that conditional independence resides among individual tests. In the present model, covariance

estimates (positive or negative) among tests in all multiple testing programs were calculated from available literature and then evaluated in the model as probability distributions (Table 6-5). Due to the lack of available data on correlations among all test permutations evaluated in the present study, the aforementioned values calculated from the existing literature were implemented for each two-test strategy. Positive and negative covariance values were modeled to influence the Se and Sp of the confirmatory test based on dependency with the initial test.

Based on herd BVDV status (positive or negative), within herd prevalence, and test strategy characteristics (diagnostic Se and Sp, test covariance), the model then determined the number of true positive (TP), false negative (FN), false positive (FP), and true negative (TN) diagnoses present within each herd iteration. Cattle of TP status (i.e. true PI animals) housed within positive herds were modeled with the probability of dying prior to weaning age<sup>1,54,55</sup> and the magnitude of a reduced weaning weight if they live (Table 3).<sup>1,56</sup> Therefore, the number of TP cattle that died prematurely was subtracted from the total number of weaned calves in both the testing and non-testing herds in year one. Current recommendations suggest that test positive cattle should be euthanized upon receipt of diagnostic test results and the model assumed this occurred.<sup>9,12</sup> The removal of TP cattle among testing herds changed herd status to BVDV negative. Therefore, herds removing all TP cattle avoided detriments to susceptible cattle (unless FN cattle were retained; see below) associated with retaining BVDV in the herd (Tables 6-2 and 6-3). Therefore, the cost of the TP calf to the testing herd was comprised of not only the individual test cost but also the loss of the projected market price that would have been realized for that respective PI animal had a diagnostic protocol not been implemented and if that calf had lived long enough to be sold (as was observed in the non-testing herd). Herds that did not test for BVDV (and therefore did not know individual animal BVDV status) did not incur the cost of euthanizing TP animals as they would have sold cattle of TP status that lived to weaning.

If FN diagnoses (i.e. PI cattle falsely classified as negative) were observed, these animals were assumed to be retained thereby allowing continued BVDV exposure to the remaining cattle of the testing herd. If FN cattle were observed, all health and production benefits for eliminating TP cattle (discussed above) were lost and morbidity, mortality, weaning weights, and cull cow percent were negatively impacted (Table 6-3) as BVDV remained in the herd (due to the presence of FN animals and continual BVDV exposure). For BVDV negative herds, no cost of TP or FN cattle was ever realized.

In accordance with current recommendations for test-positive cattle, cattle determined to be of FP diagnoses were also euthanized. Therefore the cost of FP cattle to the present herd was realized by both the test costs and the loss of income that would have been generated by that animal had that animal been correctly classified (i.e. TN status) and not euthanized. However, in contrast to TP cattle (who were modeled with the probability of being lighter at weaning), the potential market value of FP cattle equaled that of healthy cattle weaned and sold by the testing herd. Because they were euthanized, the number of FP cattle was then subtracted from the final number of weaned calves available for marketing in the testing herd only. Herds that did not test for BVDV (i.e. non-testing herds) did not incur this cost. The cost attributed to cattle correctly determined to be of TN status was modeled as the test cost of the respective test strategy. No further health or production costs to the herd were incurred by TN cattle.

In order to fully estimate the cumulative value of implementing whole-herd testing strategies in year one across a two-year timeframe, it was assumed that no BVDV testing would take place and that no replacement animals were procured in year two. Therefore, the number of breeding females for both the testing and non-testing herds entering year two was determined by subtracting cows found to be test positive (i.e. either TP or FP status which was a rare event and only determined in testing herds) and cows that were culled at the end of year one from the original number of breeding females that entered year one. The BVDV herd status in year two for herds instituting a testing strategy in year one (i.e. testing herds) was based upon the presence or absence of FN cattle remaining in the herd during year one. Conversely, the BVDV status of the non-testing herd in year two was forced to be the same as in year one as it was assumed that BVDV was carried over into year two's calf crop. The baseline calving % for both testing and non-testing herds in year two was forced to equal that of year one. However, as was assumed in year one, herds found to be BVDV positive in year two (both testing and non-testing herds) suffered the same magnitude of reproduction loss that was modeled in year one due to the presence of BVDV (Table 6-3). The magnitude of reproduction loss in year two also was forced to equal that of year one. The within-herd prevalence for positive herds in year two was forced to be equal to or less than that of year one (Table 6-2). If herd BVDV status was positive, the respective herd observed impacts in morbidity, mortality, weaning weights, and increased cull cow risk as in year one.

### ***Sensitivity Analysis***

The sensitivity of the outcome variable was analyzed for each testing strategy — herd prevalence — herd size permutation. Reported values convey the percentage of variance contributed by each respective input value to the outcome variable (i.e. the cumulative value of testing for BVDV across a two year timeframe). For each of the testing strategies, five largest contributors of variance were reported for each of the BVDV herd prevalence-herd size permutations.

### ***Application of the Model***

The model was conducted with a commercial Monte Carlo simulation program<sup>a</sup>, an add-in for a commercial software package<sup>b</sup>. Each simulation included 10,000 iterations using a fixed random number seed. Descriptive statistics (mean and standard deviation) and 95% prediction intervals were produced by the simulation program<sup>a</sup>. Iterations were captured from all models and combined into three independent data sets (by herd size) and evaluated with mixed models by a commercial statistical program<sup>c</sup>. In all models, the cumulative value of the testing strategy across both years one and two was the response variable. Fixed effects included herd BVDV status (positive or negative), BVDV testing strategy, herd BVDV prevalence, and the testing strategy by herd prevalence interaction. An alpha level of 0.05 was implemented for all analyses.

## **Results**

### ***Application of the model***

In the present study, BVDV test strategy, herd prevalence, and the test strategy by herd prevalence interaction was found to be significantly associated ( $P < 0.05$ ) with the cumulative value of testing for BVDV across a two year timeframe in U.S. cow-calf herds. Across all testing strategies and herd BVDV prevalences, the model-adjusted value of testing for BVDV for BVDV positive herds (among all herd sizes) was always a profitable venture and was significantly greater ( $P < 0.05$ ) when compared to herds of BVDV negative status (Table 6-6). Across all testing modalities, significant differences ( $P < 0.05$ ) were observed between all three herd prevalences within each respective herd size (Table 6-6). Testing for BVDV when the herd prevalence was elevated (47%) was always an economically valid practice by the second year in

each of the three herd sizes (regardless of test strategy) (Table 6-6). Conversely, lower herd prevalences (19% and 7.7%) failed to observe a profitable outcome in each of the three herd sizes regardless of testing strategy (Table 6-6).

The testing strategy by herd prevalence effect was significantly associated ( $P < 0.05$ ) with the cumulative value of implementing BVDV testing strategies across a two year timeframe for each respective herd size. These model-adjusted estimates are displayed in Table 6-7. Across all three herd sizes, Test 2 (initial test: IHC, confirmatory test: IHC) was observed to be the superior testing strategy (or did not statistically differ from Test 6 [initial test: PCR, confirmatory test: PCR] or Test 8 [initial test: ACE, confirmatory test: IHC]) in all but the lowest prevalence in the 50 head herd. In this case, Test 8 was the superior test (despite all cumulative estimates at the end of year two being of negative value in this respective scenario). Conversely, Test 3 (initial test: ACE, confirmatory test: none) displayed either the poorest or tied as the poorest performer at each herd prevalence within each respective herd size (Table 6-7). The remaining test strategies alternated ranks depending upon herd size and herd prevalence (Table 6-7). Among all herd sizes, the majority of two-test protocols displayed a positive economic outcome when the herd prevalence reached the 47% level. However, in all scenarios, single-test strategies (Tests 1, 3, and 5) failed to be observed as the superior testing modality; however, in all three herd sizes, testing strategies 1 and 5 did display positive economic outcomes when herd prevalences reached the 47% level (Table 6-7).

The cumulative value of implementing each respective testing strategy for each of the three modeled herd BVDV prevalences in a 100 head beef cow herd is graphically displayed in Figure 1. Although not statistically evaluated across herd sizes, instituting whole-herd BVDV testing strategies when herd prevalence (or the likelihood of a herd being BVDV positive) is low (19% and 7.7% in this model) is rarely an economically beneficial endeavor. Graphical observations for herd sizes of 50 and 500 cows displayed similar interpretation.

### ***Sensitivity Analysis***

Sensitivity analyses were performed for all testing strategies for each herd prevalence among all three herd sizes. For illustrative purposes, sensitivity analyses for the most favorable (Test 2 [initial test: IHC, confirmatory test: IHC]) and the poorest testing strategy (Test 3 [initial test: ACE, confirmatory test: none]) are displayed in Table 6-8. The top five contributors to the



variance of the cumulative value of testing for BVDV over a two year period are listed. For Test 2 (initial test: IHC, confirmatory test: IHC), cost of the initial test explained a vast majority of the variance across all three herd prevalences at each of the three herd sizes (Table 6-8). In Test 3 (initial test: ACE, confirmatory test: none), specificity of the test was the major contributor of variance for both all herd prevalence levels across all three herd sizes (Table 6-8).

## Discussion

The findings in the present study indicate that across all three evaluated herd sizes (50, 100, and 500 breeding females), BVDV test strategy, herd prevalence, and the test strategy by herd prevalence interaction are significantly associated ( $P < 0.05$ ) with the cumulative value of testing for BVDV across a two year timeframe in U.S. cow-calf herds. These data are relevant as they convey the extreme necessity of critically evaluating the economic constraints of considering a national BVDV eradication program as well as instituting a whole-herd BVDV testing protocol within individual cow-calf herds.

Overall, these data suggest that a positive economic outcome among U.S. cow-calf herds implementing a whole herd BVDV eradication campaign will only be realized when herd prevalence (i.e. the proportion of herds housing  $\geq 1$  PI animal) is relatively high (47% in this study). This issue is highly significant from a regulatory aspect given the concern of whether or not a national BVDV eradication program is necessary in the U.S. In Europe, numerous countries have initiated BVDV eradication programs. Herd prevalences among dairy herds in the countries of Denmark, Sweden, Norway, and Germany have been shown to vary between 25-50%.<sup>32-34,57,58</sup> In comparison, the BVDV herd prevalence in the U.S. has repeatedly been shown to be  $\leq 10\%$  indicating that only a very small number of herds would ever benefit from a whole herd testing program.<sup>1,29,30</sup> Consequently, due to the low nationwide herd BVDV prevalence, our data suggests that a national BVDV eradication program in the U.S. is not economically justified at the individual producer level.

Shifting the focus from the national herd population to individual herds, the term “herd prevalence” can be substituted with the probability of an individual herd being BVDV positive. Practicing veterinarians work with individual herds and generally have a thorough understanding of that production system; therefore, the decision to recommend a whole herd testing strategy is based on the probability that the respective herd possesses PI cattle. Although the data in the

present study suggests that herds deemed to be BVDV positive demonstrate a positive economic outcome by the second year after a testing protocol is implemented (Table 6-6), in reality the true BVDV status of a herd is typically unknown (unless a whole herd test is performed). Therefore, in lieu of a whole herd test, veterinarians assess the probability that individual herds are BVDV positive or negative based upon clinical and reproductive history, animal procurement practices, and diagnostic test results on dead cattle. Although this process is a standard method of clinical practice, previous research observed that only 19% of herds suspected of harboring PI cattle by the herd veterinarians were truly positive for PI cattle upon testing.<sup>30</sup> However, prior research has displayed that the likelihood of introducing BVDV to the resident herd across a 10 year timeframe when pregnant heifers were imported was 47% if the calves were not BVDV tested upon parturition.<sup>59</sup> Therefore, in addition to the national herd prevalence value of 7.7%, these two values (19% and 47%) constitute the herd prevalences evaluated in the present study and thereby also represent the likelihood that any given herd is BVDV positive.

Based on the findings in the present study, accurate discernment of probable herd BVDV status is imperative to avoid large financial losses attributed to the respective whole herd test (Table 6-6). For example, in herd sizes of 50, 100, and 500 cows, instituting a whole herd BVDV test based on the veterinarian's belief that the probability of the herd being positive is 0.47 when in reality the true probability is 0.19 costs the herd ~ \$400, \$800, and \$4000, respectively, on average (Table 6-6). Therefore, practicing veterinarians must have a clear understanding of the risk of being BVDV positive in each respective herd and thereby accurately categorize the probability of being BVDV positive in order to minimize negative economic outcomes.

Several factors other than the optimal economic value comprise the decision of which BVDV testing modality should be implemented.<sup>10,11</sup> Testing in series does require that a second test be performed on cattle that test positive on the initial test. Veterinarians may subsequently find that a two-test strategy may be met with some opposition by producers thereby reducing the compliance of performing the second test. Although economic benefit may not be maximized, the data in the current study suggests that numerous testing strategies (including one-time test strategies Tests 1 [initial test: IHC, confirmatory test: none] and 5 [initial test: PCR, confirmatory test: none]) are financially sensible at high herd BVDV prevalences in each of the three herd sizes (Table 6-7). Therefore, as long as herd prevalence is high or the probability of a

herd being BVDV positive is high, veterinarians can tailor their test strategy to meet the practical and logistical limits of their staff, their clientele, and their laboratory of choice. However, given the above findings, if Test 3 (initial test: ACE, confirmatory test: none) is implemented, we advise against euthanizing test positive cattle to avoid the financial loss incurred from false positive diagnoses. In these cases, isolating and feeding test positive cattle until market weight is attained can be a viable option; however, this can create some logistical and biosecurity concerns to the producer if true PI cattle are retained on the farm. In addition, if test-positive cattle are truly PI with BVDV, they may not perform as well as FP cattle and are at a greater risk of dying before they reach market weight.<sup>1,54-56</sup>

As stated above, numerous clinical indications will dictate if a whole herd test is necessary and economically justifiable. In these cases, it is essential to know which testing strategies will provide the most value to the producer. In the present study, IHC on skin tissue followed by a confirmatory IHC test on initial positives (Test 2) was consistently the superior (or one of the superior) testing strategies across herd prevalence and herd size. Therefore, not only is testing for BVDV economically justified at elevated herd prevalences, implementation of Test 2 will maximize the financial return to the producer at the herd sizes evaluated in the current study. The sensitivity analysis for this testing strategy indicates that the testing cost is the driving factor contributing to the variance in cumulative value. However, across all herd sizes, as herd prevalence increased, the cost of the test declined in rank of variation contribution. This is likely attributed to an elevation in the value of testing for BVDV due to a greater likelihood of detecting PI cattle at high herd prevalences thereby reducing the economic impacts attributed to test cost.

Conversely, a onetime ACE test on skin tissue (Test 3) performed poorly (Table 6-7) at each herd prevalence level within each herd size. The sensitivity analysis performed for this test indicates that diagnostic Sp of Test 3 is a major contributor for this result among all three herd prevalences and herd sizes evaluated. While previous estimates comprising to the distribution of diagnostic Sp used in the current study may appear adequate, in low prevalence scenarios the probability of a test positive specimen being of FP status is typically greater than that of TP. In test and cull programs, the sensitivity values indicate that false-positive diagnoses (especially at low herd prevalences) greatly impacts the value of the testing method to the producer. Only when herd BVDV prevalence levels reached 47% did diagnostic Sp become relatively

comparable to other variance contributors. This is likely attributed to the fact that at high herd prevalences, the probability that test positive cattle are truly diseased (i.e. positive predictive values) is elevated.<sup>53</sup> Conversely, diagnostic Sp for Test 2 did not contribute to the variance for this test strategy; which is likely attributed to the serial testing method implemented to reduce the likelihood of false positive diagnoses.<sup>53</sup> Therefore, in populations in which disease prevalence is low, maximizing the diagnostic Sp of the respective test protocol is necessary to minimize false-positive diagnoses. This finding is important as veterinary diagnostic laboratories are often asked to interpret test outcomes for both practicing veterinarians and their clientele. However, based on a previous survey of 26 fully accredited veterinary diagnostic laboratories in the U.S., only 55% indicated that they provide BVDV management information to veterinarians or producers who submit samples.<sup>60</sup> Additionally, of those diagnostic laboratories stating they do provide counseling, only 61% recommended confirmatory testing on cattle with positive test results.<sup>60</sup> The findings in the present study suggest that regardless of herd size, diagnostic test Sp should be a major concern to practicing veterinarians and diagnostic laboratories. Therefore, two-test strategies are optimal (although not exclusive; see below) to reduce the risk of FP diagnoses especially at low herd prevalences.

Despite the data in the present study, all findings were generated by model simulation and therefore are dependent upon the quality of the model and the information comprising model input and not upon empirical data. Although the present model was designed to incorporate the main production parameters among beef cow-calf herds in the U.S., we acknowledge that many cow-calf production systems don't follow the same model which may impact the value of BVDV testing. Therefore, it may be difficult to extrapolate our data to herds that employ other management practices (e.g. fall-calving herds). To our knowledge, the distributions in the present model reflect the available data within the veterinary literature. Where data was sparse, distributions were widened to accommodate that lack of knowledge. Therefore, as more data is accumulated, model distributions (and subsequently the outcome variable) may become more precise.

In summary, across all 12 testing strategies, our findings suggest that herd BVDV prevalence dictates the economic viability of implementing BVDV testing programs in 50, 100 and 500 head herd sizes. These findings provide evidence that when the predictive probability of a herd being BVDV positive is relatively low (0.077 and 0.19), recommending whole-herd

testing for BVDV is not financially beneficial. Therefore, given that the herd BVDV prevalence of the United States is  $\leq 10\%$ , a national BVDV eradication program is not economically justified at the producer-level. Conversely, private practitioners are urged to reserve the implementation of whole herd testing regimens for herds that are highly probable of possessing BVDV. If a whole-herd test is performed and the probability of BVDV is high, our data indicate that the value of testing can be maximized in most cases by implementing a testing strategy consisting of IHC on skin tissue as the initial test followed by a second IHC test on individuals that test positive on the first test. A onetime ACE test on skin tissue (Test 3) performed the worst of all 12 testing strategies. The sensitivity analysis for Test 3 indicated that diagnostic Sp (or probability of a FP diagnoses) was the major variance contributor to the value of the testing strategy. This suggests that financial losses were attributed to euthanizing FP cattle in herds that implemented that respective test strategy. The data presented in the present study provides evidence that the value of whole-herd BVDV testing among cow-calf herds in the U.S. must be critically analyzed prior to implementation.

## **Sources and Manufacturers**

- a. Oracle<sup>®</sup> Crystal Ball, Fusion edition, release 11.1.1.3.00 (Oracle Corp., Redwood Shores, CA, USA)
- b. Microsoft<sup>®</sup> Excel 2007 (© 1985-2007 Microsoft Corp., Redmond, WA, USA)
- c. Proc Mixed, SAS [Version 9.1], Cary, N.C.

**Table 6.1: BVDV testing modalities evaluated in the present study.**

<b>Test</b>	<b>Initial Test</b>	<b>Confirmatory Test</b>
1	IHC	none
2	IHC	IHC
3	ACE	none
4	ACE	ACE
5	PCR	none
6	PCR	PCR
7	IHC	ACE
8	ACE	IHC
9	PCR	ACE
10	PCR	IHC
11	IHC	PCR
12	ACE	PCR

**Table 6.2: Input distributions for numerous baseline herd-level factors used in the model.**

Parameter Description	Distribution	Truncate values	Source
Herd BVDV Prevalence	Bernoulli (Yes/No) (0.077/0.923) (0.19/0.81) (0.47/0.53)	0 - 1	Wittum et al 2001 Waldner et al 2008 USDA-APHIS Info Sheet 2009 Smith et al 2009 Unpublished data
Within Herd PI prevalence (Calves)*	Lognormal (0, 5.05%, 8.7%)	0.12% - 30.0%	Cornish et al 2005 Waldner et al 2008 Unpublished data
Within Herd PI prevalence (Mature Cattle)	Log-Normal (0, 0.04%, 0.05%)	0% - 30%	White et al 2007 Smith et al 2008 Gnad et al 2005
Calving %	Triangular (85.5%, 91%, 95.5%)	85.5% - 95.5%	SPA-2000 (ISU-IRM) SPA-2001 (Herds from IA, IL, and MI) SPA-2002 (Herds from IA and IL) SPA-2003 (Herds from IA, IL, KY, MI) SPA-2000 (ND/SD herds) SPA-2005 (Herds from IA and IL)
Pre-weaning Morbidity	Triangular (0%, 3.8%, 5.32%)	0% - 5.32%	NAHMS Beef Part II: 1997 Beef Cow-Calf Health & Health Management Practices
Health costs of morbid cattle (costs per hd)	Triangular (\$5.00, \$12.00, \$20.00)	\$5.00 - \$20.00	Salman, et al 1991a Salman, et al 1991b Hird, et al 1991
Pre-weaning Mortality (birth to weaning)	Triangular (2.8%, 3.5%, 4.9%)	2.8% - 4.9%	SPA-2000 (ISU-IRM) SPA-2001 (Herds from IA, IL, and MI) SPA-2002 (Herds from IA and IL) SPA-2003 (Herds from IA, IL, KY, MI) SPA-2000 (ND/SD herds) SPA-2005 (Herds from IA and IL) NAHMS Beef Part II: 1997 Beef Cow-Calf Health & Health Management Practices
Weaning weight (lbs)	Normal (530, 92)	300 - 660	USDA Beef 2007-08 Part I: Reference of Beef Cow-calf Management Practices in the United States, 2007-08
Cull Cow %	Triangular (10%, 12%, 15%)	10% - 15%	USDA Beef 1997 Part III: Reference of 1997 Beef Cow-Calf Production Management and Disease Control
Cull Cow market price	Normal (\$0.43, \$0.04)	\$0.30 - \$0.56	Iowa State University Extension-Ag Decision Maker Utility Cow Price 1999-2009

\*Within-herd BVDV prevalence was only relevant if herd BVDV status was found to be positive.



**Table 6.3: Input distributions describing the additional health effects inflicted by BVDV.**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Truncate values</b>	<b>Source</b>
Reduction in Calving % due to BVDV (conception loss, abortion, stillbirths)	Triangular (0%, 2%, 30%)	0% - 30%	Waldner et al, 2008 Kelling et al, 1990 Taylor et al, 1997 Van Campen et al 2000
Additional pre-weaning morbidity (due to retainment of PI cattle in herd)	Triangular (0%, 5%, 10%)	0% - 10%	Kelling et al 1990 Waldner et al 2008
Additional pre-weaning mortality (due to retainment of PI cattle in herd)	Triangular (0%, 1%, 10%)	0% - 10%	Waldner et al, 2008 Kelling et al, 1990 Taylor et al, 1997 Van Campen et al 2000
Assumed reduction in weaning weight (due to retainment of PI cattle in herd) (lbs)	Triangular (12.1, 28.6, 44.7)	12.1 - 44.7	Waldner et al 2008
Probability of PI cattle surviving until weaning	Triangular (0, 0.5, 1.0)	0 - 1	Houe 1992 Houe 1993 Waldner et al 2008
Assumed reduction in weaning weight of PI calf (if calf lived to weaning) (lbs)	Triangular (68, 162, 255)	68 - 180	Waldner et al 2008 Taylor et al, 1997

**Table 6.4: Input distributions regarding laboratory fees for individual BVDV diagnostic tests.**

Parameter Description	Test	Distribution	Truncate values	Source
Test Setup Fee (\$) (initial and confirmatory tests)	All testing modalities	Triangular (\$0, \$8, \$13)	\$0 - \$13	Kansas State University Iowa State University South Dakota State University Colorado State University
Test Cost (\$)	IHC	Triangular (\$4.00, \$4.33, \$5.50)	\$4.00 - \$5.50	Kansas State University Iowa State University South Dakota State University Colorado State University
	ACE	Triangular (\$4.50, \$6.00, \$9.00)	\$4.50 - \$9.00	Kansas State University Iowa State University South Dakota State University Colorado State University
	Real-time PCR	Triangular (\$5.70, \$6.00, \$6.30)	\$5.70 - \$6.30	Kansas State University

**Table 6.5: Input distributions for diagnostic tests listed in Table 3.**

Parameter Description	Test	Distribution	Truncate values	Source
Test Sensitivity	IHC	Triangular (97.6%, 99.6%, 100%)	97.6% - 100%	Thur et al 1996 Njaa et al 2000 Cornish et al 2005 Fulton et al 2006 Grooms et al 2002 Brodersen et al 2004
	ACE	Triangular (98%, 100%, 100%)	98% - 100%	Cornish et al 2005 Fulton et al 2006 Hilbe et al 2007a Kuhne et al 2005 Edmondson et al 2007
	Real-time PCR	Triangular (99%, 100%, 100%)	99% - 100%	Reinauer et al 2007
Test Specificity	IHC	Triangular (98.8%, 99.6%, 100%)	98.8% - 100%	Cornish et al 2005 Fulton et al 2006 Hilbe et al 2007a Grooms et al 2002
	ACE	Triangular (97.7%, 99.3%, 100%)	97.7% - 100%	Cornish et al 2005 Fulton et al 2006 Hilbe et al 2007a Hilbe et al 2007b Kuhne et al 2005 Edmondson et al 2007
	Real-time PCR	Triangular (99%, 100%, 100%)	99% - 100%	Reinauer et al 2007
Test Covariance (positive)	All testing modalities	Triangular (0, 0.006, 0.045)	0 - 0.045	Cornish et al 2005 Fulton et al 2006 Hilbe et al 2007a Grooms et al 2002 Hill et al 2007
Test Covariance (negative)	All testing modalities	Triangular (0, 0.019, 0.045)	0 - 0.045	Cornish et al 2005 Fulton et al 2006 Hilbe et al 2007a Grooms et al 2002 Hill et al 2007

**Table 6.6: Model-adjusted<sup>1</sup> means (and 95% confidence intervals) of the value of performing whole herd BVDV tests in U.S. cow-calf herds at herd sizes of 50, 100, and 500 head. For both independent factors (individual herd BVDV status and herd BVDV prevalence), superscripts within each column denote statistical differences at the 0.05 level.**

		Herd Size		
		50	100	500
<b>Individual Herd BVDV Status</b>	<b>Pos</b>	\$1,091.18 <sup>a</sup> (\$1,084.68, \$1,097.67)	\$2,213.57 <sup>a</sup> (\$2,201.82, \$2,225.32)	\$9,178.33 <sup>a</sup> (\$9,178.33, \$9,310.75)
	<b>Neg</b>	-\$377.63 <sup>b</sup> (-\$377.63, -\$370.21)	-\$852.64 <sup>b</sup> (-\$860.35, -\$846.92)	-\$4,533.20 <sup>b</sup> (-\$4,371.11, -\$4,495.29)
<b>Herd BVDV Prevalence</b>	<b>47%</b>	\$316.07 <sup>a</sup> (\$309.59, \$322.56)	\$593.69 <sup>a</sup> (\$581.62, \$605.77)	\$2,044.89 <sup>a</sup> (\$2,151.07, \$2,595.25)
	<b>19%</b>	-\$96.04 <sup>b</sup> (-\$102.53, -\$89.56)	-\$262.89 <sup>b</sup> (-\$274.97, -\$250.81)	-\$2,034.76 <sup>b</sup> (-\$2,034.76, -\$1,906.54)
	<b>7.7%</b>	-\$260.54 <sup>c</sup> (-\$267.02, -\$254.05)	-\$626.78 <sup>c</sup> (-\$638.86, -\$614.70)	-\$3,533.70 <sup>c</sup> (-\$3,533.70, -\$3,405.48)

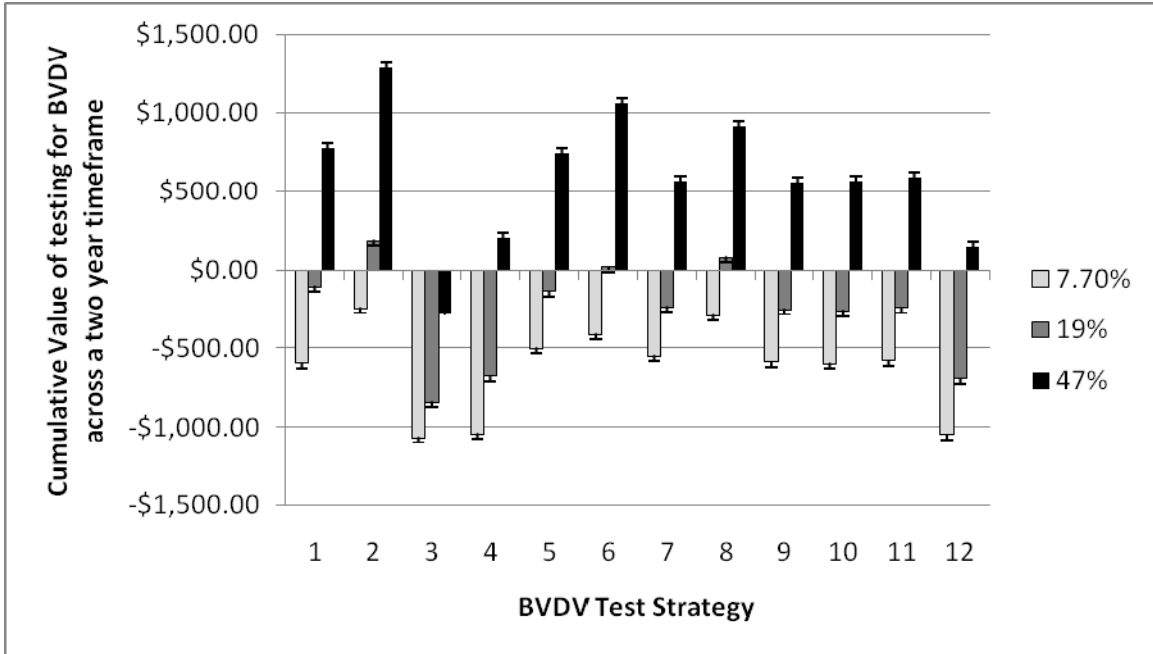
<sup>1</sup> From mixed models accounting for herd BVDV status and herd BVDV prevalence as fixed effects. Only the results of the effect of interest (value of performing whole-herd BVDV testing strategies) are reported.

**Table 6.7: Model-adjusted<sup>1</sup> means for BVDV testing strategy by herd prevalence interaction within herd sizes of 50, 100, and 500 head. Superscripts within each column denote statistical differences at the 0.05 level.**

Test	Herd Size = 50			Herd Size = 100			Herd Size = 500		
	7.7%	19%	47%	7.7%	19%	47%	7.7%	19%	47%
1	-\$179.53 <sup>c,d</sup>	\$37.75 <sup>b</sup>	\$459.03 <sup>c</sup>	-\$595.82 <sup>d</sup>	-\$105.50 <sup>d</sup>	\$776.73 <sup>d</sup>	-\$3261.06 <sup>e</sup>	-\$1667.34 <sup>d,e,f</sup>	\$2373.15 <sup>c</sup>
2	-\$120.82 <sup>b</sup>	\$90.56 <sup>a</sup>	\$658.30 <sup>a</sup>	-\$243.95 <sup>a</sup>	\$184.40 <sup>a</sup>	\$1282.02 <sup>a</sup>	-\$1446.95 <sup>a</sup>	\$116.67 <sup>a</sup>	\$4964.69 <sup>a</sup>
3	-\$498.38 <sup>f</sup>	-\$388.57 <sup>g</sup>	-\$107.77 <sup>i</sup>	-\$1067.41 <sup>e</sup>	-\$840.55 <sup>g</sup>	-\$282.60 <sup>h</sup>	-\$5684.86 <sup>f</sup>	-\$4777.30 <sup>i</sup>	-\$1955.80 <sup>g</sup>
4	-\$484.09 <sup>f</sup>	-\$301.58 <sup>f</sup>	\$143.45 <sup>g</sup>	-\$1045.09 <sup>e</sup>	-\$677.09 <sup>f</sup>	\$205.84 <sup>f</sup>	-\$603.782 <sup>f</sup>	-\$4017.16 <sup>h</sup>	-\$87.11 <sup>e</sup>
5	-\$198.89 <sup>d</sup>	-\$44.30 <sup>d,e</sup>	\$406.72 <sup>d</sup>	-\$501.39 <sup>c</sup>	-\$134.69 <sup>d</sup>	\$743.67 <sup>d</sup>	-\$2900.77 <sup>d</sup>	-\$1050.18 <sup>c</sup>	\$3588.99 <sup>b</sup>
6	-\$202.48 <sup>d</sup>	-\$6.30 <sup>c</sup>	\$542.19 <sup>b</sup>	-\$414.07 <sup>b</sup>	\$18.30 <sup>c</sup>	\$1061.33 <sup>b</sup>	-\$2093.85 <sup>c</sup>	\$56.00 <sup>a</sup>	\$5155.07 <sup>a</sup>
7	-\$174.28 <sup>c,d</sup>	-\$10.95 <sup>c</sup>	\$349.01 <sup>e</sup>	-\$549.98 <sup>c,d</sup>	-\$234.21 <sup>e</sup>	\$563.74 <sup>e</sup>	-\$3297.32 <sup>e</sup>	-\$1988.23 <sup>g</sup>	\$1237.38 <sup>d</sup>
8	-\$58.58 <sup>a</sup>	\$57.83 <sup>b</sup>	\$443.97 <sup>c</sup>	-\$292.66 <sup>a</sup>	\$79.15 <sup>b</sup>	\$912.85 <sup>c</sup>	-\$1585.53 <sup>a,b</sup>	-\$525.91 <sup>b</sup>	\$3463.03 <sup>b</sup>
9	-\$242.92 <sup>e</sup>	-\$73.88 <sup>e</sup>	\$312.29 <sup>f</sup>	-\$585.68 <sup>d</sup>	-\$252.80 <sup>e</sup>	\$560.95 <sup>e</sup>	-\$3241.53 <sup>e</sup>	-\$1654.88 <sup>d,e,f</sup>	\$2406.56 <sup>c</sup>
10	-\$249.49 <sup>e</sup>	-\$65.76 <sup>e</sup>	\$327.23 <sup>e,f</sup>	-\$599.14 <sup>d</sup>	-\$261.81 <sup>e</sup>	564.98 <sup>e</sup>	-\$3280.52 <sup>e</sup>	-\$1811.21 <sup>e,f,g</sup>	\$2530.38 <sup>c</sup>
11	-\$162.98 <sup>c</sup>	-\$28.91 <sup>c,d</sup>	\$390.82 <sup>d</sup>	-\$577.83 <sup>d</sup>	-\$238.87 <sup>e</sup>	\$591.10 <sup>e</sup>	-\$3441.33 <sup>e</sup>	-\$2111.94 <sup>g</sup>	\$1305.67 <sup>d</sup>
12	-\$495.88 <sup>f</sup>	-\$328.64 <sup>f</sup>	\$93.74 <sup>h</sup>	-\$1048.30 <sup>e</sup>	-\$690.99 <sup>f</sup>	\$143.69 <sup>g</sup>	-\$5797.50 <sup>f</sup>	-\$4216.31 <sup>h</sup>	-\$443.33 <sup>f</sup>

<sup>1</sup> From mixed models accounting for test strategy, herd BVDV prevalence, and the test strategy by herd BVDV prevalence as fixed effects. Only the results of the effect of interest (value of performing whole-herd BVDV testing strategies) are reported.

**Figure 6.1: Graphical display of the value of implementing whole-herd BVDV tests for each of the 12 BVDV testing strategies in a 100 cow beef herd at each of the three BVDV herd prevalences evaluated in the present study. Error bars denote 95% confidence intervals.**



**Table 6.8: Sensitivity analysis for Test 2 (initial test: IHC, confirmatory test: IHC) and Test 3 (initial test: ACE, confirmatory test: none) at each respective herd size and herd BVDV prevalence. Within each column, the top five contributors to variance are displayed. The values reflect the individual percentage of variation of the outcome variable (cumulative value of testing for BVDV across a two year timeframe).**

Test	Variable	Herd Size = 50			Herd Size = 100			Herd Size = 500		
		7.70%	19%	47%	7.70%	19%	47%	7.70%	19%	47%
2	Cost of initial Test	-55.2%	-53.1%	-23.6%	-56.4%	-53.6%	-26.1%	-55.8%	-53.2%	-27.7%
	Preweaning Morbidity	-31.2%	-30.3%	-16.1%	-29.6%	-29.5%	-12.3%	-30.4%	-29.9%	-13.3%
	Health Costs of Morbid cattle	-13.1%	-13.2%	-5.8%	-13.4%	-13.6%	--	-12.9%	-13.4%	-7.6%
	Pre-weaning mortality	-0.1%	--	--	--	--	--	--	--	--
	Additional preweaning mortality due to BVDV	0.2%	1.3%	29.2%	0.1%	1.4%	25.8%	0.2%	0.9%	17.0%
	Weaning Weight	--	0.9%	13.4%	0.1%	0.5%	15.7%	--	--	12.5%
	Cull Cow Market Value	--	--	--	--	--	-6.7%	--	--	--
	Within-herd BVDV prevalence	--	--	--	--	--	--	-0.1%	-0.9%	-11.9%
3	Specificity of initial test	69.4%	69.1%	28.7%	60.6%	59.1%	26.9%	86.5%	83.9%	34.2%
	Cost of initial Test	-27.7%	-22.5%	-9.1%	-28.6%	-26.3%	-7.9%	-8.2%	-7.5%	--
	Additional preweaning mortality due to BVDV	0.3%	3.4%	21.9%	0.5%	3.6%	26.7%	0.3%	1.7%	20.1%
	Reduction in Calving % due to BVDV	0.3%	1.0%	--	--	--	6.1%	--	--	4.8%
	Weaning Weight	-1.3%	--	--	-5.6%	-5.4%	--	-4.4%	-2.2%	--
	Within herd BVDV prevalence in Year 1	--	-1.8%	-14.6%	-0.4%	-2.5%	-17.7	-0.4%	-2.5%	-22.6%
	Probability of a PI calf surviving until weaning	--	--	-9.5%	--	--	--	--	--	--
	Within herd BVDV prevalence in Year 2	--	--	--	--	--	--	--	--	-7.1%

## **CHAPTER 7 - Dissertation Conclusion**

Current veterinary literature has repeatedly displayed the complexity and unpredictability of BVDV. These characteristics have provided researchers the opportunity to observe a high degree of volatility in beef production when BVDV is present. The sources of this variability are likely broad but our work has proposed that one factor may be the quantity of virus shed by individual PI cattle. In addition, BVDV strain differences, the level of herd immunity, and the degree of stress placed on susceptible cattle (among others) likely play a role in the development of clinical disease among cattle exposed to BVDV. Given that PI cattle are the likely reservoir of the virus, BVDV control programs are highly focused at identifying (and removing) PI cattle (diagnostic tests), elevating herd immunity (vaccines), and preventing introduction of BVDV to the resident cow-calf herd (biosecurity). Given these widely adopted tools, prevention and control of BVDV can be effectively performed and our data would suggest that producer education has benefited this endeavor. However, from a diagnostic test standpoint, our data infers that herd prevalence, or the probability of a herd being BVDV positive, should be relatively high in order to economically justify a whole-herd testing strategy. Therefore, due to the low BVDV herd prevalence among U.S. beef cow-calf herds, a nationwide BVDV eradication program implemented within this sector of the U.S. beef industry is not a rational approach to reducing the risk of BVDV to beef production systems. Alternatively, veterinarians should evaluate each herd for its likelihood of housing PI cattle; the decision to implement a diagnostic test strategy is then based on those findings.



## CHAPTER 8 - Bibliography

1. Olafson P, MacCallum AD, Fox FH. An Apparently New Transmissible Disease of Cattle. *New York State Veterinary Medical Association*. Syracuse, N.Y., 1946;205-213.
2. Kelling CL, Stine LC, Rump KK, et al. Investigation of bovine viral diarrhea virus infections in a range beef cattle herd. *J Am Vet Med Assoc* 1990;197:589-593.
3. Wittum TE, Grotelueschen DM, Brock KV, et al. Persistent bovine viral diarrhoea virus infection in US beef herds. *Prev Vet Med* 2001;49:83-94.
4. Loneragan GH, Thomson DU, Montgomery DL, et al. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. *J Am Vet Med Assoc* 2005;226:595-601.
5. O'Connor AM, Sorden SD, Apley MD. Association between the existence of calves persistently infected with bovine viral diarrhea virus and commingling on pen morbidity in feedlot cattle. *Am J Vet Res* 2005;66:2130-2134.
6. Guichon PT, Haines D, Jim GK, et al. Investigation of the Role of Bovine Viral Diarrhea Virus (BVDV) in Undifferentiated Fever of Feedlot Cattle. *AABP online proceeding* 2006;39:272-274.
7. Booker CW, Abutarbush SM, Morley PS, et al. The effect of bovine viral diarrhea virus infections on health and performance of feedlot cattle. *Can Vet J* 2008;49:253-260.
8. Waldner CL, Kennedy RI. Associations between health and productivity in cow-calf beef herds and persistent infection with bovine viral diarrhea virus, antibodies against bovine viral diarrhea virus, or antibodies against infectious bovine rhinotracheitis virus in calves. *Am J Vet Res* 2008;69:916-927.
9. Hessman BE, Fulton RW, Sjeklocha DB, et al. Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. *Am J Vet Res* 2009;70:73-85.
10. Ramsey FK, Chivers WH. Mucosal Disease of Cattle. *The North American Veterinarian* 1953:629-633.
11. Nielsen SW, Horney FD, Hulland TJ, et al. Mucosal Disease Of Cattle In Ontario. *Can J Comp Med Vet Sci* 1955;19:318-324.
12. Dow C, Jarrett W, McIntyre W. A Disease of Cattle in Britain Resembling the Virus Diarrhea-Mucosal Disease Complex. *Vet Rec* 1956;68:293-296.
13. Swope RE, Luedke AJ. A Mucosal Disease in Cattle in Pennsylvania. *JAVMA* 1956:111-115.
14. Olafson P, Richard CG. Further Observations on the Virus Diarrhea (new Transmissible Disease) of Cattle. *Cornell Vet* 1947;37:104-106.
15. Seibold HR. The Pathology of Mucosal Disease in Alabama. *JAVMA* 1956:21-26.
16. Hoag WG, Rooney JR, Williams WJ. A Mucosal-Type Disease in Cattle in Virginia. *JAVMA* 1956:105-110.
17. Olson C, Hoerlein AB. Observations on Mucosal Disease of Cattle. *JAVMA* 1956:466-470.
18. Baker JA, York CJ, Gillepsie JH, et al. Virus diarrhea in cattle. *Am J Vet Res* 1954;15:525-531.

19. Huck RA. Mucosal disease complex. *J Comp Pathol* 1957;67:267-276.
20. Pritchard WR. The Bovine Viral Diarrhea-Mucosal Disease Complex. *Adv Vet Sci Comp Med* 1963;41:1-47.
21. Kendrick JW, Kronlund N. The Effect of Bovine Virus Diarrhea Virus on Pregnant Cows. 6th Internat Cong Anim Reprod & Artificial Insemination 1968;541-543.
22. Kendrick JW. Bovine viral diarrhea-mucosal disease virus infection in pregnant cows. *Am J Vet Res* 1971;32:533-544.
23. Casaro AP, Kendrick JW, Kennedy PC. Response of the bovine fetus to bovine viral diarrhea-mucosal disease virus. *Am J Vet Res* 1971;32:1543-1562.
24. Kniazeff AJ, Rimer V, Gaeta L. Gamma-globulin foetal bovine sera: significance in virology. *Nature* 1967;214:805-806.
25. Gillespie JH. The Isolation of Noncytopathic Virus Diarrhea Virus from Two Aborted Bovine Fetuses. *Cornell Vet* 1967;57:564-571.
26. Gratzek JB. In Discussion on Comments of Bovine Viral Diarrhea-Mucosal Disease. *JAVMA* 1968;152:768-770.
27. Ward GM, Roberts SJ, McEntee K, et al. A study of experimentally induced bovine viral diarrhea-mucosal disease in pregnant cows and their progeny. *Cornell Vet* 1969;59:525-538.
28. Fray MD, Mann GE, Bleach EC, et al. Modulation of sex hormone secretion in cows by acute infection with bovine viral diarrhoea virus. *Reproduction* 2002;123:281-289.
29. Fray MD, Mann GE, Clarke MC, et al. Bovine viral diarrhea virus: its effects on estradiol, progesterone and prostaglandin secretion in the cow. *Theriogenology* 1999;51:1533-1546.
30. Grooms DL, Brock KV, Pate JL, et al. Changes in ovarian follicles following acute infection with bovine viral diarrhea virus. *Theriogenology* 1998;49:595-605.
31. Kafi M, McGowan MR, Kirkland PD, et al. The effect of bovine pestivirus infection on the superovulatory response of Friesian heifers. *Theriogenology* 1997;48:985-996.
32. McGowan MR, Kirkland PD, Richards SG, et al. Increased reproductive losses in cattle infected with bovine pestivirus around the time of insemination. *Vet Rec* 1993;133:39-43.
33. Houe H, Pedersen KM, Meyling A. The effect of bovine virus diarrhoea virus infection on conception rate. *Prev Vet Med* 1993;15:117-123.
34. Virakul P, Fahning ML, Joo HS, et al. Fertility of cows challenged with a cytopathic strain of Bovine Viral Diarrhea virus during an outbreak of spontaneous infection with a noncytopathic strain. *Theriogenology* 1988;29:441-449.
35. Whitmore HL, Zemjanis R, Olson J. Effect of bovine viral diarrhea virus on conception in cattle. *J Am Vet Med Assoc* 1981;178:1065-1067.
36. Ssentongo YK, Johnson RH, Smith JR. Association of bovine viral diarrhoea-mucosal disease virus with ovaritis in cattle. *Aust Vet J* 1980;56:272-273.
37. Brownlie J, Clarke MC, Howard CJ. Experimental infection of cattle in early pregnancy with a cytopathic strain of bovine virus diarrhoea virus. *Res Vet Sci* 1989;46:307-311.
38. Liess B, Orban S, Frey HR, et al. Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine virus in cattle. II. Inoculation of pregnant cows without detectable neutralizing antibodies to BVD virus 90-229 days before parturition (51st to 190th day of gestation). *Zentralbl Veterinarmed B* 1984;31:669-681.
39. Done JT, Terlecki S, Richardson C, et al. Bovine virus diarrhoea-mucosal disease virus: pathogenicity for the fetal calf following maternal infection. *Vet Rec* 1980;106:473-479.

40. Wilson TM, deLahunta A, Confer L. Cerebellar degeneration in dairy calves: clinical, pathologic, and serologic features of an epizootic caused by bovine viral diarrhea virus. *J Am Vet Med Assoc* 1983;183:544-547.
41. Scott FW, Kahrs RF, De Lahunte A, et al. Virus induced congenital anomalies of the bovine fetus. I. Cerebellar degeneration (hypoplasia), ocular lesions and fetal mummification following experimental infection with bovine viral diarrhea-mucosal disease virus. *Cornell Vet* 1973;63:536-560.
42. Kahrs RF, Scott FW, de Lahunte A. Congenital cerebella hypoplasia and ocular defects in calves following bovine viral diarrhea-mucosal disease infection in pregnant cattle. *J Am Vet Med Assoc* 1970;156:1443-1450.
43. Kahrs RF, Scott FW, de Lahunta A. Bovine viral diarrhea-mucosal disease, abortion, and congenital cerebellar hypoplasia in a dairy herd. *J Am Vet Med Assoc* 1970;156:851-857.
44. Badman RT, Mitchell G, Jones RT, et al. Association of bovine viral diarrhoea virus infection to hydranencephaly and other central nervous system lesions in perinatal calves. *Aust Vet J* 1981;57:306-307.
45. Hewicker-Trautwein M, Trautwein G. Porencephaly, hydranencephaly and leukoencephalopathy in ovine fetuses following transplacental infection with bovine virus diarrhoea virus: distribution of viral antigen and characterization of cellular response. *Acta Neuropathol* 1994;87:385-397.
46. Binkhorst GJ, Journee DL, Wouda W, et al. Neurological disorders, virus persistence and hypomyelination in calves due to intra-uterine infections with bovine virus diarrhoea virus. I. Clinical symptoms and morphological lesions. *Vet Q* 1983;5:145-155.
47. Bielefeldt Ohmann H. An oculo-cerebellar syndrome caused by congenital bovine viral diarrhoea virus-infection. *Acta Vet Scand* 1984;25:36-49.
48. Wohrmann T, Hewicker-Trautwein M, Fernandez A, et al. Distribution of bovine virus diarrhoea viral antigens in the central nervous system of cattle with various congenital manifestations. *Zentralbl Veterinarmed B* 1992;39:599-609.
49. Brown TT, De Lahunte A, Scott FW, et al. Virus induced congenital anomalies of the bovine fetus. II. Histopathology of cerebellar degeneration (hypoplasia) induced by the virus of bovine viral diarrhea-mucosal disease. *Cornell Vet* 1973;63:561-578.
50. Scott FW, Kahrs RF, Parsonson IM. A cytopathogenic strain of bovine viral diarrhea-mucosal disease virus isolated from a bovine fetus. *Cornell Vet* 1972;62:74-84.
51. Duffell SJ, Sharp MW, Winkler CE, et al. Bovine virus diarrhoea-mucosal disease virus-induced fetopathy in cattle: Efficacy of prophylactic maternal pre-exposure. *Vet Rec* 1984;114:558-561.
52. Baker JC. Bovine viral diarrhea virus: a review. *J Am Vet Med Assoc* 1987;190:1449-1458.
53. Johnson DW, Muscoplat CC. Immunologic abnormalities in calves with chronic bovine viral diarrhea. *Am J Vet Res* 1973;34:1139-1141.
54. Malmquist WA. Bovine viral diarrhea-mucosal disease: Etiology, pathogenesis, and applied immunity. *JAVMA* 1968;152:763-768.
55. Coria MF, McClurkin AW. Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhea virus. *J Am Vet Med Assoc* 1978;172:449-451.
56. McClurkin AW, Littledike ET, Cutlip RC, et al. Production of cattle immunotolerant to bovine viral diarrhea virus. *Can J Comp Med* 1984;48:156-161.
57. Huck RA. A Mucosal Disease of Cattle. *Vet Rec* 1957;69:1207-1215.

58. Thomson RG, Savan M. Studies on Virus Diarrhea and Mucosal Disease of Cattle. *Can J Comp Med Vet Sci* 1963;27:207-214.
59. Steck F, Lazary S, Fey H, et al. Immune responsiveness in cattle fatally affected by bovine virus diarrhea-mucosal disease. *Zentralbl Veterinarmed B* 1980;27:429-445.
60. Roeder PL, Drew TW. Mucosal disease of cattle: a late sequel to fetal infection. *Vet Rec* 1984;114:309-313.
61. Brownlie J, Clarke MC, Howard CJ. Experimental production of fatal mucosal disease in cattle. *Vet Rec* 1984;114:535-536.
62. Bolin SR, McClurkin AW, Cutlip RC, et al. Severe clinical disease induced in cattle persistently infected with noncytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. *Am J Vet Res* 1985;46:573-576.
63. Brownlie J, Clarke MC, Howard CJ, et al. Pathogenesis and epidemiology of bovine virus diarrhoea virus infection of cattle. *Ann Rech Vet* 1987;18:157-166.
64. Brock KV, Redman DR, Vickers ML, et al. Quantitation of bovine viral diarrhea virus in embryo transfer flush fluids collected from a persistently infected heifer. *J Vet Diagn Invest* 1991;3:99-100.
65. Fulton RW, Hessman B, Johnson BJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *J Am Vet Med Assoc* 2006;228:578-584.
66. Fulton RW, Briggs RE, Ridpath JF, et al. Transmission of Bovine viral diarrhea virus 1b to susceptible and vaccinated calves by exposure to persistently infected calves. *Can J Vet Res* 2005;69:161-169.
67. Fulton RW, Johnson BJ, Briggs RE, et al. Challenge with Bovine viral diarrhea virus by exposure to persistently infected calves: protection by vaccination and negative results of antigen testing in nonvaccinated acutely infected calves. *Can J Vet Res* 2006;70:121-127.
68. Patel JR, Shilleto RW, Williams J, et al. Prevention of transplacental infection of bovine foetus by bovine viral diarrhoea virus through vaccination. *Arch Virol* 2002;147:2453-2463.
69. Moerman A, Straver PJ, de Jong MC, et al. A long term epidemiological study of bovine viral diarrhoea infections in a large herd of dairy cattle. *Vet Rec* 1993;132:622-626.
70. Collins ME, Heaney J, Thomas CJ, et al. Infectivity of pestivirus following persistence of acute infection. *Vet Microbiol* 2009;138:289-296.
71. Givens MD, Heath AM, Brock KV, et al. Detection of bovine viral diarrhea virus in semen obtained after inoculation of seronegative postpubertal bulls. *Am J Vet Res* 2003;64:428-434.
72. Givens MD, Riddell KP, Walz PH, et al. Noncytopathic bovine viral diarrhea virus can persist in testicular tissue after vaccination of peri-pubertal bulls but prevents subsequent infection. *Vaccine* 2007;25:867-876.
73. Duncan C, Ridpath J, Palmer MV, et al. Histopathologic and immunohistochemical findings in two white-tailed deer fawns persistently infected with Bovine viral diarrhea virus. *J Vet Diagn Invest* 2008;20:289-296.
74. Duncan C, Van Campen H, Soto S, et al. Persistent Bovine viral diarrhea virus infection in wild cervids of Colorado. *J Vet Diagn Invest* 2008;20:650-653.
75. Passler T, Walz PH, Ditchkoff SS, et al. Evaluation of hunter-harvested white-tailed deer for evidence of bovine viral diarrhea virus infection in Alabama. *J Vet Diagn Invest* 2008;20:79-82.

76. Ridpath JF, Mark CS, Chase CC, et al. Febrile response and decrease in circulating lymphocytes following acute infection of white-tailed deer fawns with either a bvdv1 or a bvdv2 strain. *J Wildl Dis* 2007;43:653-659.
77. Tarry DW, Bernal L, Edwards S. Transmission of bovine virus diarrhoea virus by blood feeding flies. *Vet Rec* 1991;128:82-84.
78. Lang-Ree JR, Vatn T, Kommisrud E, et al. Transmission of bovine viral diarrhoea virus by rectal examination. *Vet Rec* 1994;135:412-413.
79. Gunn HM. Role of fomites and flies in the transmission of bovine viral diarrhoea virus. *Vet Rec* 1993;132:584-585.
80. Mars MH, Brusckke CJ, van Oirschot JT. Airborne transmission of BHV1, BRSV, and BVDV among cattle is possible under experimental conditions. *Vet Microbiol* 1999;66:197-207.
81. Houe H, Baker JC, Maes RK, et al. Prevalence of cattle persistently infected with bovine viral diarrhoea virus in 20 dairy herds in two counties in central Michigan and comparison of prevalence of antibody-positive cattle among herds with different infection and vaccination status. *J Vet Diagn Invest* 1995;7:321-326.
82. Grooms DL, Kaiser L, Walz PH, et al. Study of cattle persistently infected with bovine viral diarrhoea virus that lack detectable virus in serum. *J Am Vet Med Assoc* 2001;219:629-631.
83. Larson RL, Miller RB, Kleiboeker SB, et al. Economic costs associated with two testing strategies for screening feeder calves for persistent infection with bovine viral diarrhoea virus. *J Am Vet Med Assoc* 2005;226:249-254.
84. Bolin SR, McClurkin AW, Coria MF. Frequency of persistent bovine viral diarrhoea virus infection in selected cattle herds. *Am J Vet Res* 1985;46:2385-2387.
85. Gnad DP, Walz P, Sargeant JM, et al. Prevalence of bovine viral diarrhoea virus in young purebred beef bulls in Kansas. *Bov Pract* 2005;39:101-104.
86. White BJ, Larson RL, Thomson DU. Mature Open Cows are Rarely Persistently Infected with Bovine Viral Diarrhoea Virus. *Beef Cattle Research*: Kansas State University, 2007.
87. Smith RL, Sanderson MW, Walz PH, et al. Sensitivity of polymerase chain reaction for detection of bovine viral diarrhoea virus in pooled serum samples and use of pooled polymerase chain reaction to determine prevalence of bovine viral diarrhoea virus in auction market cattle. *J Vet Diagn Invest* 2008;20:75-78.
88. Houe H. Age distribution of animals persistently infected with bovine virus diarrhoea virus in twenty-two Danish dairy herds. *Can J Vet Res* 1992;56:194-198.
89. Cutlip RC, McClurkin AW, Coria MF. Lesions in clinically healthy cattle persistently infected with the virus of bovine viral diarrhoea--glomerulonephritis and encephalitis. *Am J Vet Res* 1980;41:1938-1941.
90. Houe H. Survivorship of animals persistently infected with bovine virus diarrhoea virus. *Prev Vet Med* 1993;15:275-283.
91. Duffell SJ, Harkness JW. Bovine virus diarrhoea-mucosal disease infection in cattle. *Vet Rec* 1985;117:240-245.
92. Bezek DM, Mechor GD. Identification and eradication of bovine viral diarrhoea virus in a persistently infected dairy herd. *J Am Vet Med Assoc* 1992;201:580-586.
93. Stevens E, Thomson DU. Effects of testing and removal of persistently infected bovine viral diarrhoea virus feeder calves on morbidity and mortality of home pen-associated feeder calves. *The Bovine Practitioner* 2006:307.
94. Holland RE, Bezek DM, Sprecher DJ, et al. Investigation of an epizootic of bovine viral diarrhoea virus infection in calves. *J Am Vet Med Assoc* 1993;202:1849-1854.

95. Taylor LF, Van Donkersgoed J, Radostits OM, et al. Investigation of an outbreak of mucosal disease in a beef cattle herd in southwestern Saskatchewan. *Can Vet J* 1994;35:425-432.
96. Taylor LF, Janzen ED, Van Donkersgoed J. Losses over a 2-year period associated with fetal infection with the bovine viral diarrhoea virus in a beef cow-calf herd in Saskatchewan. *Can Vet J* 1997;38:23-28.
97. Waldner CL, Campbell JR. Use of serologic evaluation for antibodies against bovine viral diarrhoea virus for detection of persistently infected calves in beef herds. *Am J Vet Res* 2005;66:825-834.
98. Waldner CL. Serological status for N. caninum, bovine viral diarrhoea virus, and infectious bovine rhinotracheitis virus at pregnancy testing and reproductive performance in beef herds. *Anim Reprod Sci* 2005;90:219-242.
99. Daly RF, Neiger RD. Outbreak of Salmonella enterica serotype Newport in a beef cow-calf herd associated with exposure to bovine viral diarrhoea virus. *J Am Vet Med Assoc* 2008;233:618-623.
100. Elam NA, Thomson DU, Gleghorn JF. Effects of long- or short-term exposure to a calf identified as persistently infected with bovine viral diarrhoea virus on feedlot performance of freshly weaned, transport-stressed, beef heifers. *J Anim Sci* 2008.
101. Rebhun WC, French TW, Perdrizet JA, et al. Thrombocytopenia associated with acute bovine virus diarrhoea infection in cattle. *J Vet Intern Med* 1989;3:42-46.
102. Corapi WV, Elliott RD, French TW, et al. Thrombocytopenia and hemorrhages in veal calves infected with bovine viral diarrhoea virus. *J Am Vet Med Assoc* 1990;196:590-596.
103. Corapi WV, French TW, Dubovi EJ. Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhoea virus. *J Virol* 1989;63:3934-3943.
104. Bolin SR, Ridpath JF. Differences in virulence between two noncytopathic bovine viral diarrhoea viruses in calves. *Am J Vet Res* 1992;53:2157-2163.
105. Pellerin C, van den Hurk J, Lecomte J, et al. Identification of a new group of bovine viral diarrhoea virus strains associated with severe outbreaks and high mortalities. *Virology* 1994;203:260-268.
106. Ridpath JF, Bolin SR, Dubovi EJ. Segregation of bovine viral diarrhoea virus into genotypes. *Virology* 1994;205:66-74.
107. Fulton RW, Cook BJ, Step DL, et al. Evaluation of health status of calves and the impact on feedlot performance: assessment of a retained ownership program for postweaning calves. *Can J Vet Res* 2002;66:173-180.
108. Fulton RW, Ridpath JF, Saliki JT, et al. Bovine viral diarrhoea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. *Can J Vet Res* 2002;66:181-190.
109. Fulton RW, Ridpath JF, Confer AW, et al. Bovine viral diarrhoea virus antigenic diversity: impact on disease and vaccination programmes. *Biologicals* 2003;31:89-95.
110. Fulton RW, Saliki JT, Confer AW, et al. Bovine viral diarrhoea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. *J Vet Diagn Invest* 2000;12:33-38.
111. Obando C, Baule C, Pedrique C, et al. Serological and molecular diagnosis of bovine viral diarrhoea virus and evidence of other viral infections in dairy calves with respiratory disease in Venezuela. *Acta Vet Scand* 1999;40:253-262.
112. Baker JC. The clinical manifestations of bovine viral diarrhoea infection. *Vet Clin North Am Food Anim Pract* 1995;11:425-445.

113. Fairbanks KK, Rinehart CL, Ohnesorge WC, et al. Evaluation of fetal protection against experimental infection with type 1 and type 2 bovine viral diarrhoea virus after vaccination of the dam with a bivalent modified-live virus vaccine. *J Am Vet Med Assoc* 2004;225:1898-1904.
114. Walz PH, Bell TG, Wells JL, et al. Relationship between degree of viremia and disease manifestation in calves with experimentally induced bovine viral diarrhoea virus infection. *Am J Vet Res* 2001;62:1095-1103.
115. Vogel GL, Parrott JC. Mortality survey in feedyards. The incidence of death from digestive, respiratory and other causes in feedyards in the Great Plains. *Comp Con Ed Pract Vet* 1994;16:227-234.
116. Smith RA. Effects of feedlot disease on economics, production and carcass value. *AABP* 2000;125-128.
117. Roeber DL, Speer NC, Gentry JG, et al. Feeder Cattle Health Management: Effects on Morbidity Rates, Feedlot Performance, Carcass Characteristics, and Beef Palatability. *The Professional Animal Scientist* 2001;17:39-44.
118. Duff GC, Galyean ML. BOARD-INVITED REVIEW: Recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* %R 102527/jas2006-501 2006;jas.2006-2501.
119. Jim K. Impact of bovine respiratory disease (BRD) from the perspective of the Canadian beef producer. *Anim Health Res Rev* 2009;10:109-110.
120. Booker CW, Abutarbush SM, Morley PS, et al. Microbiological and histopathological findings in cases of fatal bovine respiratory disease of feedlot cattle in Western Canada. *Can Vet J* 2008;49:473-481.
121. Reggiardo C. Role BVD virus in shipping fever of feedlot cattle. Case studies and diagnostic considerations. Proc of the 22nd Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians 1979;315-320.
122. Gagea MI, Bateman KG, van Dreumel T, et al. Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diagn Invest* 2006;18:18-28.
123. Radostits OM, Townsend HG. The controversy surrounding the role of the bovine virus diarrhoea virus (BVDV) in the pathogenesis of pneumonic pasteurellosis in cattle. *Proc Am Assoc Bov Pract* 1989;111-114.
124. Pottgieter LND. Immunosuppression of cattle as a result of bovine viral diarrhoea infection. *Agri Pract* 1988;9:7-14.
125. Peterhans E, Jungi TW, Schweizer M. BVDV and innate immunity. *Biologicals* 2003;31:107-112.
126. Chase CC, Elmowalid G, Yousif AA. The immune response to bovine viral diarrhoea virus: a constantly changing picture. *Vet Clin North Am Food Anim Pract* 2004;20:95-114.
127. Fulton RW, Purdy CW, Confer AW, et al. Bovine viral diarrhoea viral infections in feeder calves with respiratory disease: interactions with *Pasteurella* spp., parainfluenza-3 virus, and bovine respiratory syncytial virus. *Can J Vet Res* 2000;64:151-159.
128. Penny CD, Low JC, Nettleton PF, et al. Concurrent bovine viral diarrhoea virus and *Salmonella typhimurium* DT104 infection in a group of pregnant dairy heifers. *Vet Rec* 1996;138:485-489.
129. Potgieter LN, McCracken MD, Hopkins FM, et al. Effect of bovine viral diarrhoea virus infection on the distribution of infectious bovine rhinotracheitis virus in calves. *Am J Vet Res* 1984;45:687-690.

130. Gratzek JB, Peter CP, Ramsey FK. Isolation and characterization of a strain of infectious bovine rhinotracheitis virus associated with enteritis in cattle: isolation, serologic characterization, and induction of the experimental disease. *Am J Vet Res* 1966;27:1567-1572.
131. Reggiardo C, Kaeberle ML. Detection of bacteremia in cattle inoculated with bovine viral diarrhea virus. *Am J Vet Res* 1981;42:218-221.
132. Potgieter LN, McCracken MD, Hopkins FM, et al. Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. *Am J Vet Res* 1984;45:1582-1585.
133. Potgieter LN, McCracken MD, Hopkins FM, et al. Comparison of the pneumopathogenicity of two strains of bovine viral diarrhea virus. *Am J Vet Res* 1985;46:151-153.
134. Richer L, Marois P, Lamontagne L. Association of Bovine Viral Diarrhea Virus with Multiple Viral Infections in Bovine Respiratory Disease Outbreaks. *Can Vet J* 1988;29:713-717.
135. Wheat JD, McKercher DG, York CJ. Virus Diarrhea in California. *The California Veterinarian* 1954;November-December:26-35.
136. Gillespie JH, Baker JA, Mc EK. A cytopathogenic strain of virus diarrhea virus. *Cornell Vet* 1960;50:73-79.
137. Bolin SR, Littledike ET, Ridpath JF. Serologic detection and practical consequences of antigenic diversity among bovine viral diarrhea viruses in a vaccinated herd. *Am J Vet Res* 1991;52:1033-1037.
138. Potgieter LND. Immunosuppression of cattle as a result of bovine viral diarrhea infection. *Agri Pract* 1988;9:7-14.
139. Bolin SR, Grooms DL. Origination and consequences of bovine viral diarrhea virus diversity. *Vet Clin North Am Food Anim Pract* 2004;20:51-68.
140. Ridpath JF. Practical significance of heterogeneity among BVDV strains: impact of biotype and genotype on U.S. control programs. *Prev Vet Med* 2005;72:17-30; discussion 215-219.
141. Larson RL, Pierce VL, Grotelueschen DM, et al. Economic Evaluation of Beef Cowherd Screening for Cattle Persistently-infected with Bovine Viral Diarrhea Virus. *The Bovine Practitioner* 2002;36.
142. Grooms DL, Givens MD, Sanderson MW, et al. Integrated BVD Control Plans for Beef Operations. *The Bovine Practitioner* 2009;43:106-116.
143. Larson RL, Brodersen BW, Grotelueschen DM, et al. Considerations for Bovine Viral Diarrhea (BVD) Testing. *The Bovine Practitioner* 2005;39:96-100.
144. Brodersen BW. Immunohistochemistry used as a screening method for persistent bovine viral diarrhea virus infection. *Vet Clin North Am Food Anim Pract* 2004;20:85-93.
145. Cornish TE, van Olphen AL, Cavender JL, et al. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J Vet Diagn Invest* 2005;17:110-117.
146. Hilbe M, Arquint A, Schaller P, et al. Immunohistochemical diagnosis of persistent infection with Bovine Viral Diarrhea Virus (BVDV) on skin biopsies. *Schweiz Arch Tierheilkd* 2007;149:337-344.
147. Grooms DL, Keilen ED. Screening of neonatal calves for persistent infection with bovine viral diarrhea virus by immunohistochemistry on skin biopsy samples. *Clin Diagn Lab Immunol* 2002;9:898-900.



148. Hilbe M, Stalder H, Peterhans E, et al. Comparison of five diagnostic methods for detecting bovine viral diarrhoea virus infection in calves. *J Vet Diagn Invest* 2007;28-34.
149. Kennedy JA. Diagnostic efficacy of a reverse transcriptase-polymerase chain reaction assay to screen cattle for persistent bovine viral diarrhoea virus infection. *J Am Vet Med Assoc* 2006;229:1472-1474.
150. Kennedy JA, Mortimer RG, Powers B. Reverse transcription-polymerase chain reaction on pooled samples to detect bovine viral diarrhoea virus by using fresh ear-notch-sample supernatants. *J Vet Diagn Invest* 2006;18:89-93.
151. Reinauer S, Moessler G, Schoder G, et al. Evaluation of the AnDiaTec Bovir real-time RT-PCR kit for the detection of BVDV in pooled bovine ear notch and blood samples. *AAVLD Annual Conference Proceedings* 2007:66.
152. Larson RL, Grotelueschen DM, Brock KV, et al. BVD decision/ management guidelines for beef veterinarians. *The Bovine Practitioner* 2004;38:103-104.
153. Larson RL, Grotelueschen DM, Brock KV, et al. Bovine viral diarrhoea (BVD): review for beef cattle veterinarians. *The Bovine Practitioner* 2004;38:93-102.
154. USDA. Persistent Infection of Calves with Bovine Viral Diarrhoea Virus on U.S. Beef Cow-calf Operations In: USDA:APHIS:VS C, ed. Fort Collins, CO, 2009.
155. Smith RL, Sanderson MW, Renter DG, et al. A stochastic model to assess the risk of introduction of bovine viral diarrhoea virus to beef cow-calf herds. *Prev Vet Med* 2008.
156. Sanderson MW. Modeling introduction risk and impact of bovine viral diarrhoea virus in cow-calf herds. *Proc Academy of Vet Consultants* 2007;37-51.
157. Sanderson MW, Dargatz DA, Garry FB. Biosecurity practices of beef cow-calf producers. *J Am Vet Med Assoc* 2000;217:185-189.
158. Sanderson MW, Gay JM. Veterinary involvement in management practices of beef cow-calf producers. *J Am Vet Med Assoc* 1996;208:488-491.
159. Cortese VS, Grooms DL, Ellis J, et al. Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhoea virus type 1 by use of a modified-live virus vaccine. *Am J Vet Res* 1998;59:1409-1413.
160. Cortese VS, West KH, Hassard LE, et al. Clinical and immunologic responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhoea virus. *J Am Vet Med Assoc* 1998;213:1312-1319.
161. Dean HJ, Hunsaker BD, Bailey OD, et al. Prevention of persistent infection in calves by vaccination of dams with noncytopathic type-1 modified-live bovine viral diarrhoea virus prior to breeding. *Am J Vet Res* 2003;64:530-537.
162. Brock KV, McCarty K, Chase CC, et al. Protection against fetal infection with either bovine viral diarrhoea virus type 1 or type 2 using a noncytopathic type 1 modified-live virus vaccine. *Vet Ther* 2006;7:27-34.
163. Ficken MD, Ellsworth MA, Tucker CM, et al. Effects of modified-live bovine viral diarrhoea virus vaccines containing either type 1 or types 1 and 2 BVDV on heifers and their offspring after challenge with noncytopathic type 2 BVDV during gestation. *J Am Vet Med Assoc* 2006;228:1559-1564.
164. Miksch D. Preconditioning programs for feeder cattle. *Mod Vet Pract* 1984;65:341-344.
165. Galyean ML, Perino LJ, Duff GC. Interaction of cattle health/immunity and nutrition. *J Anim Sci* 1999;77:1120-1134.

166. Duff GC, Malcolm-Callis KJ, Walker DA, et al. Effects of intranasal versus intramuscular modified live vaccines and vaccine timing on health and performance by newly received beef cattle. *Bovine Practitioner* 2000;34:66-71.
167. Duff GC, Galyean ML. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* 2007;85:823-840.
168. Fairbanks K, Schnackel J, Chase CC. Evaluation of a modified live virus type-1a bovine viral diarrhoea virus vaccine (Singer strain) against a type-2 (strain 890) challenge. *Vet Ther* 2003;4:24-34.
169. Ellis J, West K, Cortese V, et al. Effect of maternal antibodies on induction and persistence of vaccine-induced immune responses against bovine viral diarrhoea virus type II in young calves. *J Am Vet Med Assoc* 2001;219:351-356.
170. Dean HJ, Leyh R. Cross-protective efficacy of a bovine viral diarrhoea virus (BVDV) type 1 vaccine against BVDV type 2 challenge. *Vaccine* 1999;17:1117-1124.
171. Kelling CL, Hunsaker BD, Steffen DJ, et al. Characterization of protection against systemic infection and disease from experimental bovine viral diarrhoea virus type 2 infection by use of a modified-live noncytopathic type 1 vaccine in calves. *Am J Vet Res* 2007;68:788-796.
172. Van Campen H, Vorpahl P, Huzurbazar S, et al. A case report: evidence for type 2 bovine viral diarrhoea virus (BVDV)-associated disease in beef herds vaccinated with a modified-live type 1 BVDV vaccine. *J Vet Diagn Invest* 2000;12:263-265.
173. Ellsworth MA, Fairbanks KK, Behan S, et al. Fetal protection following exposure to calves persistently infected with bovine viral diarrhoea virus type 2 sixteen months after primary vaccination of the dams. *Vet Ther* 2006;7:295-304.
174. Zimmerman AD, Boots RE, Valli JL, et al. Evaluation of protection against virulent bovine viral diarrhoea virus type 2 in calves that had maternal antibodies and were vaccinated with a modified-live vaccine. *J Am Vet Med Assoc* 2006;228:1757-1761.
175. Rodning SP, Marley MS, Zhang Y, et al. Comparison of three commercial vaccines for preventing persistent infection with bovine viral diarrhoea virus. *Theriogenology*.
176. Grooms DL, Bolin SR, Coe PH, et al. Fetal protection against continual exposure to bovine viral diarrhoea virus following administration of a vaccine containing an inactivated bovine viral diarrhoea virus fraction to cattle. *Am J Vet Res* 2007;68:1417-1422.
177. Givens MD, Waldrop JG. Bovine viral diarrhoea virus in embryo and semen production systems. *Vet Clin North Am Food Anim Pract* 2004;20:21-38.
178. Walz PH, Givens MD, Cochran A, et al. Effect of dexamethasone administration on bulls with a localized testicular infection with bovine viral diarrhoea virus. *Can J Vet Res* 2008;72:56-62.
179. Howard TH, Bean B, Hillman R, et al. Surveillance for persistent bovine viral diarrhoea virus infection in four artificial insemination centers. *J Am Vet Med Assoc* 1990;196:1951-1955.
180. Bridges V. Risk analysis - an introduction and its application in APHIS:VS. 102nd Annu Meet US Anim Health Assoc 1998;242-247.
181. Vose D. *Risk Analysis: A quantitative guide*. 2nd ed: John Wiley & Sons Ltd, 2000.
182. Rae AG, Sinclair JA, Nettleton PF. Survival of bovine virus diarrhoea virus in blood from persistently infected cattle. *Vet Rec* 1987;120:504.
183. Brock KV, Grooms DL, Ridpath J, et al. Changes in levels of viremia in cattle persistently infected with bovine viral diarrhoea virus. *J Vet Diagn Invest* 1998;10:22-26.

184. Confer AW, Fulton RW, Step DL, et al. Viral antigen distribution in the respiratory tract of cattle persistently infected with bovine viral diarrhoea virus subtype 2a. *Vet Pathol* 2005;42:192-199.
185. Callan RJ, Garry FB. Biosecurity and bovine respiratory disease. *Vet Clin North Am Food Anim Pract* 2002;18:57-77.
186. Njaa B, Clark EG, Janzen ED, et al. Diagnosis of persistent bovine viral diarrhoea virus infection by immunohistochemical staining of formalin-fixed skin biopsy specimens. *J Vet Diagn Invest* 2000;12:393-399.
187. Brodersen B, White AK, Smith D. Immunohistochemical test on skin biopsies as a method for detection of cattle persistently infected with bovine viral diarrhoea virus. *Bov Pract* 1998;31:246.
188. Thomson DU, White BJ. Backgrounding beef cattle. *Vet Clin North Am Food Anim Pract* 2006;22:373-398.
189. Finney DJ. The Spearman Karber Method. In *Statistical Method in Biological Assay*. 1952:524-530.
190. Fulton RW, Hessman BE, Ridpath JF, et al. Multiple diagnostic tests to identify cattle with Bovine viral diarrhoea virus and duration of positive test results in persistently infected cattle. *Can J Vet Res* 2009;73:117-124.
191. Bolin SR, McClurkin AW, Cutlip RC, et al. Response of cattle persistently infected with noncytopathic bovine viral diarrhoea virus to vaccination for bovine viral diarrhoea and to subsequent challenge exposure with cytopathic bovine viral diarrhoea virus. *Am J Vet Res* 1985;46:2467-2470.
192. Bolin SR, Sacks JM, Crowder SV. Frequency of association of noncytopathic bovine viral diarrhoea virus with mononuclear leukocytes from persistently infected cattle. *Am J Vet Res* 1987;48:1441-1445.
193. Fulton RW, Step DL, Ridpath JF, et al. Response of calves persistently infected with noncytopathic bovine viral diarrhoea virus (BVDV) subtype 1b after vaccination with heterologous BVDV strains in modified live virus vaccines and Mannheimia haemolytica bacterin-toxoid. *Vaccine* 2003;21:2980-2985.
194. Palfi V, Houe H, Philipsen J. Studies on the decline of bovine virus diarrhoea virus (BVDV) maternal antibodies and detectability of BVDV in persistently infected calves. *Acta Veterinaria Scandinavica* 1993;34:105-107.
195. Thiry E, Saliki J, Bublot M, et al. Reactivation of infectious bovine rhinotracheitis virus by transport. *Comp Immunol Microbiol Infect Dis* 1987;10:59-63.
196. Noisakran S, Halford WP, Veress L, et al. Role of the hypothalamic pituitary adrenal axis and IL-6 in stress-induced reactivation of latent herpes simplex virus type 1. *J Immunol* 1998;160:5441-5447.
197. Mi W, Young CR, Storts RW, et al. Restraint stress facilitates systemic dissemination of Theiler's virus and alters its pathogenicity. *Microb Pathog* 2006;41:133-143.
198. Kelling CL. Evolution of bovine viral diarrhoea virus vaccines. *Vet Clin North Am Food Anim Pract* 2004;20:115-129.
199. Erickson GA, Landgraf JG, Wessman SJ, et al. Detection and elimination of adventitious agents in continuous cell lines. *Dev Biol Stand* 1989;70:59-66.
200. Wessman SJ, Levings RL. Benefits and risks due to animal serum used in cell culture production. *Dev Biol Stand* 1999;99:3-8.

201. Vilcek S. Identification of pestiviruses contaminating cell lines and fetal calf sera. *Acta Virol* 2001;45:81-86.
202. Rossi CR, Bridgman CR, Kiesel GK. Viral contamination of bovine fetal lung cultures and bovine fetal serum. *Am J Vet Res* 1980;41:1680-1681.
203. Wellemans G, Van Opdenbosch E. [Demonstration of BVD virus (bovine diarrhea virus) in many cell lines]. *Ann Rech Vet* 1987;18:99-102.
204. Bolin SR, Matthews PJ, Ridpath JF. Methods for detection and frequency of contamination of fetal calf serum with bovine viral diarrhea virus and antibodies against bovine viral diarrhea virus. *J Vet Diagn Invest* 1991;3:199-203.
205. Levings RL, Wessman SJ. Bovine viral diarrhea virus contamination of nutrient serum, cell cultures and viral vaccines. *Dev Biol Stand* 1991;75:177-181.
206. Bolin SR, Ridpath JF. Prevalence of bovine viral diarrhea virus genotypes and antibody against those viral genotypes in fetal bovine serum. *J Vet Diagn Invest* 1998;10:135-139.
207. Nickell J, White B, Larson R, et al. Comparison of Short-Term Health and Performance Effects Related to Prophylactic Administration of Tulathromycin versus Tilmicosin in Long-Hauled, Highly Stressed Beef Stocker Calves. *Vet Ther* 2008;9:147-156.
208. OIE Terrestrial Manual 2008:698-711.
209. Ridpath JF, Bolin SR. Differentiation of types 1a, 1b and 2 bovine viral diarrhoea virus (BVDV) by PCR. *Mol Cell Probes* 1998;12:101-106.
210. Barkema HW, Bartels CJ, van Wuijckhuise L, et al. [Outbreak of bovine virus diarrhea on Dutch dairy farms induced by a bovine herpesvirus 1 marker vaccine contaminated with bovine virus diarrhea virus type 2.]. *Tijdschr Diergeneeskd* 2001;126:158-165.
211. Falcone E, Cordioli P, Tarantino M, et al. Experimental infection of calves with bovine viral diarrhoea virus type-2 (BVDV-2) isolated from a contaminated vaccine. *Vet Res Commun* 2003;27:577-589.
212. Kelling CL, Hunsaker BD, Steffen DJ, et al. Characterization of protection from systemic infection and disease by use of a modified-live noncytopathic bovine viral diarrhea virus type 1 vaccine in experimentally infected calves. *Am J Vet Res* 2005;66:1785-1791.
213. Muller-Doblies D, Arquint A, Schaller P, et al. Innate immune responses of calves during transient infection with a noncytopathic strain of bovine viral diarrhea virus. *Clin Diagn Lab Immunol* 2004;11:302-312.
214. Booker CW, Guichon PT, Jim GK, et al. Seroepidemiology of undifferentiated fever in feedlot calves in western Canada. *Can Vet J* 1999;40:40-48.
215. Dohoo I, Martin W, Stryhm H. Screening and Diagnostic Tests. *Veterinary Epidemiologic Research* Charlottetown, Prince Edward Island: AVC Inc., 2003;85-113.
216. Kleiboeker SB, Lee SM, Jones CA, et al. Evaluation of shedding of bovine herpesvirus 1, bovine viral diarrhea virus 1, and bovine viral diarrhea virus 2 after vaccination of calves with a multivalent modified-live virus vaccine. *J Am Vet Med Assoc* 2003;222:1399-1403.
217. Cortese V, Ellis J, Whittaker R, et al. BVD Virus Transmission Following Attenuated Vaccines to BVDV Seronegative Cattle. *Large Animal Practitioner* 1997;18:18-24.
218. Bolin SR, Ridpath JF. Specificity of neutralizing and precipitating antibodies induced in healthy calves by monovalent modified-live bovine viral diarrhea virus vaccines. *Am J Vet Res* 1989;50:817-821.
219. Grooms DL, Givens MD, Sanderson MW, et al. Integrated BVD control plans for beef operations. *Bov Pract* 2009;43:106-116.

220. Munoz-Zanzi CA, Johnson WO, Thurmond MC, et al. Pooled-sample testing as a herd-screening tool for detection of bovine viral diarrhoea virus persistently infected cattle. *J Vet Diagn Invest* 2000;12:195-203.
221. Schunicht OC, Booker CW, Jim GK, et al. Comparison of a multivalent viral vaccine program versus a univalent viral vaccine program on animal health, feedlot performance, and carcass characteristics of feedlot calves. *Can Vet J* 2003;44:43-50.
222. Peters AR, Thevasagayam SJ, Wiseman A, et al. Duration of immunity of a quadrivalent vaccine against respiratory diseases caused by BHV-1, PI3V, BVDV, and BRSV in experimentally infected calves. *Prev Vet Med* 2004;66:63-77.
223. Cherry BR, Reeves MJ, Smith G. Evaluation of bovine viral diarrhoea virus control using a mathematical model of infection dynamics. *Prev Vet Med* 1998;33:91-108.
224. Ridpath JF, Hietala SK, Sorden S, et al. Evaluation of the reverse transcription-polymerase chain reaction/probe test of serum samples and immunohistochemistry of skin sections for detection of acute bovine viral diarrhoea infections. *J Vet Diagn Invest* 2002;14:303-307.
225. Martin SW, Bateman KG, Shewen PE, et al. The frequency, distribution and effects of antibodies, to seven putative respiratory pathogens, on respiratory disease and weight gain in feedlot calves in Ontario. *Can J Vet Res* 1989;53:355-362.
226. Martin SW, Bateman KG, Shewen PE, et al. A group level analysis of the associations between antibodies to seven putative pathogens and respiratory disease and weight gain in Ontario feedlot calves. *Can J Vet Res* 1990;54:337-342.
227. O'Connor AM. A seroepidemiological investigation of undifferentiated bovine respiratory disease University of Guelph, 2000;201.
228. Martin SW, Bohac JG. The association between serological titers in infectious bovine rhinotracheitis virus, bovine virus diarrhoea virus, parainfluenza-3 virus, respiratory syncytial virus and treatment for respiratory disease in Ontario feedlot calves. *Can J Vet Res* 1986;50:351-358.
229. Fulton RW, Ridpath JF, Ore S, et al. Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. *Vet Microbiol* 2005;111:35-40.
230. Bolin SR. The current understanding about the pathogenesis and clinical forms of BVD. *Veterinary medicine* 1990;October:1124-1132.
231. Brock KV. The many faces of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 2004;20:1-3.
232. Brock KV. Strategies for the control and prevention of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 2004;20:171-180.
233. Peck C, Patterson DJ, Harboc M. 2009 Montana BVD-PI Herd Biosecurity Project. Bozeman, MT, 2009.
234. USDA. Census of Agriculture; United States summary and state data. *Geographical Area Series*. Part 51 ed: National Agriculture Statistics Service, 2007.
235. USDA. Biosecurity on U.S. Beef Cow-calf Operations In: USDA:APHIS:VS, ed. Fort Collins, CO, 2009.
236. Ficken MD, Ellsworth MA, Tucker CM. Evaluation of the efficacy of a modified-live combination vaccine against bovine viral diarrhoea virus types 1 and 2 challenge exposures in a one-year duration-of-immunity fetal protection study. *Vet Ther* 2006;7:283-294.

237. Fourichon C, L'Hotel L, Frappat B, et al. Farmers motivations and obstacles to enter in and comply to a voluntary BVDV control plan (abstr). *7th European Society for Veterinary Virology Pestivirus Symposium*, 2008;106.
238. Thur B, Ehrensperger F. Immunohistochemical detection of bovine viral diarrhoea virus in skin biopsies: a reliable and fast diagnostic tool. *J Vet Med* 1996;43:163-166.
239. Brodersen BW. Immunohistochemistry used as a screening method for persistent bovine viral diarrhoea virus infection. *Vet Clin Food Anim* 2004;20:85-93.
240. Kuhne S, Schroeder C, Holmquist G, et al. Detection of Bovine Viral Diarrhoea Virus Infected Cattle - Testing Tissue Samples Derived from Ear Tagging Using an Erns Capture ELISA. *Journal of Veterinary Medicine Series B* 2005;52:272-277.
241. Edmondson M, Givens MD, Walz PH, et al. Comparison of tests for detection of bovine viral diarrhoea virus in diagnostic samples. *J Vet Diagn Invest* 2007;19:376-381.
242. Niskanen R, Alenius S, Larsson B, et al. Determination of level of antibodies to bovine virus diarrhoea virus (BVDV) in bulk tank milk as a tool in the diagnosis and prophylaxis of BVDV infections in dairy herds. *Arch Virol Suppl* 1991;3:245-251.
243. Alenius S, Jacobsson SO, Cafaro E. Frequency of bovine viral diarrhoea virus infections in Sweden among heifers selected for artificial insemination. *Proceedings of the 14th World Congress on Disease* 1986;204-207.
244. Bitsch V, Ronsholt L. Control of bovine viral diarrhoea virus infection without vaccines. *Vet Clin North Am Food Anim Pract* 1995;11:627-640.
245. Loken T, Krogsrud J, Larsen IL. Pestivirus infections in Norway. Serological investigations in cattle, sheep and pigs. *Acta Vet Scand* 1991;32:27-34.
246. USDA. Beef 2007–08, Part III: Changes in the U.S. Beef Cow-Calf Industry, 1993–2008 In: USDA:APHIS:VS C, ed. Fort Collins, CO, 2008.
247. Gardner IA, Stryhn H, Lind P, et al. Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. *Prev Vet Med* 2000;45:107-122.
248. Taylor LF, Janzen ED, Ellis JA, et al. Performance, survival, necropsy, and virological findings from calves persistently infected with the bovine viral diarrhoea virus originating from a single Saskatchewan beef herd. *Can Vet J* 1997;38:29-37.
249. Lawrence J. Historic Cattle Prices. *Iowa State University Extension-Ag Decision Maker*. Ames, IA, 2009.
250. USDA. Part II: Reference of 1997 Beef Cow-Calf Health & Health Management Practices In: USDA:APHIS:VS, ed. Fort Collins, CO: National Animal Health Monitoring System, 1997.
251. Salman MD, King ME, Odde KG, et al. Annual costs associated with disease incidence and prevention in Colorado cow-calf herds participating in rounds 2 and 3 of the National Animal Health Monitoring System from 1986 to 1988. *J Am Vet Med Assoc* 1991;198:968-973.
252. Salman MD, King ME, Odde KG, et al. Annual disease incidence in Colorado cow-calf herds participating in rounds 2 and 3 of the National Animal Health Monitoring System from 1986 to 1988. *J Am Vet Med Assoc* 1991;198:962-967.
253. Hird DW, Weigler BJ, Salman MD, et al. Expenditures for veterinary services and other costs of disease and disease prevention in 57 California beef herds in the National Animal Health Monitoring System (1988-1989). *J Am Vet Med Assoc* 1991;198:554-558.
254. SPA. 2000 Summary: Iowa Beef Cow Business Record In: Center IB, ed. Ames, IA: Iowa State University Extensions, 2000.

255. SPA. 2000 North and South Dakota Herds ISU Veterinary Medicine Class VCS 436X In: Center IB, ed. Ames, IA: Iowa State University Extension, 2000.
256. SPA. 2001 SPA Summary-Iowa, Illinois, Kentucky, and Michigan In: Center IB, ed. Ames, IA: Iowa State University Extension, 2001.
257. SPA. 2002 SPA Summary - Iowa & Illinois Combined In: Center IB, ed. Ames, IA: Iowa State University Extension, 2002.
258. SPA. 2003 SPA Summary-Iowa, Illinois, Kentucky, and Michigan In: Center IB, ed. Ames, IA: Iowa State University Extension, 2003.
259. SPA. 2005 SPA Summary - Iowa & Illinois Combined In: Center IB, ed. Ames, IA: Iowa State University Extension, 2005.
260. USDA. Beef 2007–08, Part I: Reference of Beef Cow-calf Management Practices in the United States, 2007–08 In: USDA:APHIS:VS, ed. Fort Collins, Co, 2008.
261. USDA. Part III: Reference of 1997 Beef Cow-Calf Production Management and Disease Control. Fort Collins, CO: National Animal Health Monitoring Service, 1997.
262. Houe H, Meyling A. Prevalence of bovine virus diarrhoea (BVD) in 19 Danish dairy herds and estimation of incidence of infection in early pregnancy. *Prev Vet Med* 1991;11:1-16.
263. Niskanen R. Relationship between the levels of antibodies to bovine viral diarrhoea virus in bulk tank milk and the prevalence of cows exposed to the virus. *Vet Rec* 1993;133:341-344.
264. Smith RL, Sanderson MW, Renter DG, et al. A stochastic model to assess the risk of introduction of bovine viral diarrhoea virus to beef cow-calf herds. *Prev Vet Med* 2009.