

BIOSECURITY AND RISK ANALYSIS FOR COW-CALF ENTERPRISES: A SIMULATION  
MODEL FOR BOVINE VIRAL DIARRHEA VIRUS

by

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A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Clinical Sciences  
College of Veterinary Medicine

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2007

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## **Abstract**

A Monte Carlo model was developed to determine the cost-effectiveness of different biosecurity strategies for Bovine Viral Diarrhea Virus (BVDV) on cow-calf farms. Where possible, risk distributions were defined in the course of a critical literature review covering all publications since 1990 relevant to BVDV on cow-calf farms. The prevalence of persistent infections (PIs) in adult cows was unknown, so a survey of viremia in 2,990 adult cows for sale in the Midwest during 2006 was performed; prevalence was calculated to be 0.07%. In order to validate a newly developed RT-nPCR for pooled serum used for the survey, sensitivity was determined based on 100 known viremic serum samples; sensitivity was 95%, with no detectable effect of strain type.

A Monte Carlo model was developed to calculate the risk of introducing BVDV to a cow-calf herd and number of PIs introduced in one year, based on herd imports and biosecurity strategies. The results of that model were integrated with a stochastic SIR model for the spread and impact of BVDV through a cow-calf herd over 10 years, based on herd size and control strategies. The resulting model was integrated with a stochastic model for the cost of both the biosecurity and control measures used and the financial impact of BVDV infection on the herd over 10 years. The lowest risk option of 14 biosecurity strategies were calculated for 400-, 100-, and 50-head herds with 8 different import profiles, and management factors that increase financial risk due to BVDV were determined.

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# **CHAPTER 1 - A critical literature review of bovine viral diarrhea virus risk of introduction and spread on US cow-calf farms**

## **Abstract**

Bovine Viral Diarrhea Virus is a major source of economic loss in the cattle production industry. Sources of infection introduction for cow-calf producers are primarily imported cattle and infected contact herds. Importation risk may be decreased by testing and quarantine schemes, while vaccination can decrease spread after initial infection. The impact of infection on the cow-calf herd includes reproductive effects and increased calf morbidity and mortality. Identifying the risk factors leading to herd infection allows control strategies to be designed. Establishing infection dynamics, impacts, and mitigation factors permit risk analysis models to evaluate those strategies.

## **Introduction**

Bovine viral diarrhea (BVD) was first described in 1946 (Olafson et al., 1946) as a pathogen of cattle, causing diarrhea, abortion, and immunosuppression leading to respiratory disease. The disease is caused by Bovine Viral Diarrhea Virus (BVDV), a single-stranded RNA virus in the family Flaviviridae. Transmission occurs via secretion in nasolacrimal fluid and other bodily fluids. All ages are susceptible, but symptoms and outcomes vary; adults primarily exhibit reproductive effects, while youngstock experience the bulk of morbidity and mortality. In addition, calves infected between 40 and 125 days gestation with noncytopathic virus may become persistently infected (PI) (Stokstad and Loken, 2002), resulting in persistent shedding of virus in large quantities.

The BVD virus causes major production losses in the cattle industry and is endemic in much of the world. The exceptions to this are several European countries with advanced eradication programs. Success in control and eradication approaches, especially in Scandinavia (Valle et al., 2005; Hult and Lindberg, 2005), have shown that such programs are effective. In order to develop a useful control program for BVDV in the cow-calf industry, it is necessary to understand the components of the disease risk and the means of controlling those risk factors.

Risk analyses have proved a valuable tool in identifying critical control points and the economic benefits of various disease interventions (Valle et al., 2005). The purpose of this critical literature review is to collate and present pertinent information towards the development of a risk analysis model for BVD introduction and transmission within US beef cow-calf industry.

## **Methods**

A systematic literature review was performed using the CAB, Pub-Med, and Agricola databases for all journal articles between 1990 and the present referencing the keywords BVD, BVDV, bovine viral diarrhoea, and bovine viral diarrhoea. The search period was bracketed at 1990 because understanding of the virus and its mode of action was limited previous to that time and the research performed was considered to be of little use for the present purposes. Pertinent journal articles presenting original research, obtainable by the Kansas State University library system and its partner system, and written in English, Spanish, or French, have been critically reviewed. The information obtained has been compiled to provide an accurate picture of the current state of knowledge of the epidemiology of BVD in beef cattle. In addition, a few journal articles that precede 1990 were included to provide necessary background information. Gaps in the knowledge base were identified for further study. References were reviewed critically by a single reader, using checklists developed for each study type. Quality was assessed by the number of applicable checkpoints met.

## **Results**

### ***Sources of BVD Infection***

Prevention of BVDV introduction to beef cow-calf farms currently free of the virus, as well as for preventing re-introduction during control programs, requires identification of sources of infection and the risk associated with each. Although there are many sources of virus introduction documented in the literature, the majority of the risk comes from a few main sources, as described below.

Numerous successful control policies have established imported cattle by as the primary risk factor for introduction of BVDV to a herd (Valle et al., 1999; Schaik et al., 2002; Obritzhauser et al., 2005; Solis-Calderon et al., 2005). Imported cattle may be persistently infected (PI) animals (Confer et al., 2005) or animals pregnant with PI fetuses (Bitsch et al.,

2000), either of which adds a constant source of BVDV to the herd population, making high levels of transmission and chronic herd infection (Cherry et al., 1998) a possibility. PI animals are highly viremic in most cases, although they may not consistently shed high amounts of virus (Waxweiler et al., 1991). Transiently infected (TI) animals may introduce BVDV to a herd (Moen et al., 2005), but the viremias are low in contrast to PI animals so TI animals are often considered to be a low comparative risk for BVD importation (Niskanen et al., 2002b).

(Valle et al., 1999) found importation to be non-significant in herd BVDV infection status, but this study was performed near the end stages of a nation-wide eradication scheme and may have been biased by the successfully lowered prevalence in the general population. In the US, where a nationwide control policy does not exist, the advantage of importing from known negative herds is unavailable. Barring a closed herd, then, the best way to control importation risk has been assumed to be effective testing and quarantine (Hult and Lindberg, 2005; Joly et al., 2005).

Although the primary source of BVDV introduction seems to be imported animals, other sources of virus are possible. The spread of BVDV from neighboring herds is also a major concern, especially in herds on pasture (Houe et al., 1995a; Valle et al., 2000). Communal pasture use is a high risk activity (Braun et al., 1999; Valle et al., 1999; Bitsch et al., 2000; Schaik et al., 2002; Obritzhauser et al., 2005; Siegwart et al., 2006) unless the status of the other herds is known to be BVDV-negative. In effect, sharing pasture with an infected herd is equivalent to having infection within one's own herd, as free mixing of animals allows PI animals direct contact without regards for ownership. One model of disease risk, in a European setting, found that herds which shared pasture with other herds were 5.1 times as likely to be seropositive for BVD as those who did not share pasture (Valle et al., 1999).

Fenceline contact with neighboring herds is also a risk for BVDV introduction (Desilets et al., 1996; Valle et al., 1999; Bitsch et al., 2000; Campen et al., 2000; Ross, 2003), as direct contact between cattle can occur through fences and on the occasion of fence breaks. No published studies of fenceline contact risk are applicable to US cow-calf farms, but anecdotal evidence points to infection of some few herds via fenceline contact.

Other possible sources of BVDV infection, less commonly considered, include semen (Paton et al., 1990; Kirkland et al., 1991; Voges et al., 1998; Niskanen et al., 2002a), embryo transfer (Brock et al., 1991; Tsuboi and Imada, 1998), contaminated vaccine (Falcone et al.,

2003), and environmental contamination (Niskanen and Lindberg, 2003; Lindberg et al., 2004). Semen may be a risk factor if artificial insemination (AI) bulls are infected, as was found to be true in 1990 (Howard et al., 1990) at 4 AI centers in the US. However, testing procedures at AI centers are likely to have been implemented in the years since that study, and the risk is almost certainly very small. Likewise, embryo transfer was a risk factor in the past, but embryo washing techniques suggested by the International Embryo Transfer Society (, 1998) limit the risk. Vaccine is a possible source of virus when prepared with serum from PI fetuses (Bolin et al., 1991), but testing of fetal bovine serum lots by the veterinary biologics industry prevents contamination. Environmental infection has been observed only when the environment was reinhabited after short time periods, and so may be controlled by vacancy periods. Fomites such as rectal sleeves (Lang-Ree et al., 1994), needles and nose-tongs (Gunn, 1993), contaminated bottles used for injectables (Niskanen and Lindberg, 2003; Katholm and Houe, 2006), and clothing (Schaik et al., 2002) are also considered to be capable of transmitting BVDV to susceptible animals. However, most of these infection sources required the close presence of PI animals to provide recent contamination; others may be controlled through basic biosecurity measures such as cleaning and disinfection, foot baths, protective clothing for farm visitors, and appropriate use of fresh rectal sleeves, medicine bottles, and needles for each farm.

Other animals may also be infected by the virus. Species found to carry BVDV or a related virus naturally or experimentally include wild ruminants, such as deer (Corn et al., 1990; Braun et al., 1998; Uttenthal et al., 2005), and other livestock species such as llamas (Carman et al., 2005; Foster et al., 2005; Mattson et al., 2006), swine (Liess and Moennig, 1990), and bison (Deregt et al., 2005). No transmission of BVDV from other infected species to cattle has been validated, although cross-species transmission has not been disproven. The risk of transmission from wild ruminants is completely unknown, as no data exist as to the prevalence of BVDV in these populations or their capability in transmitting the virus. BVDV has also been detected in some insects, such as face flies (Gunn, 1993), and transmission from biting flies has been confirmed in a laboratory setting (Tarry et al., 1991). Controls necessary to decrease infection from fenceline contact with neighboring herds, such as double fences and avoiding adjacent pastures during the risk period, likely would be efficacious against insect and other livestock sources as well.

### ***Prevalence of BVDV***

The risk of introducing BVD by way of imported cattle, the primary risk noted above, depends on virus prevalence in the cattle imported. The prevalence varies between age groups of cattle (Hietala et al., 2001), as PI animals experience higher rates of mortality and lower life expectancy (Houe, 1993). Younger animals will represent a higher risk of PI introduction, as will pregnant animals through the prevalence of persistent infection of the fetus *in utero*. Within herd management practices of the source herd also may impact import risk, as herds with good control strategies are less likely to be infected. In the various control and eradication programs in Europe, sourcing animals from certified BVDV negative herds has enabled safe purchase (Valle et al., 2005; Hult and Lindberg, 2005; Joly et al., 2005) and communal grazing (Rossmanith et al., 2005) of animals.

Variation in prevalence estimation requires knowledge of the specific class and source of cattle tested and the diagnostic test used. There are studies estimating prevalence of BVDV in feeder calves (Taylor et al., 1995; Fulton et al., 2000; Loneragen et al., 2005) and bulls (Howard et al., 1990; Cleveland, 2003; Givens et al., 2003b; Gnad et al., 2005), as well as herd prevalence (Wittum et al., 1997; Wittum et al., 2001), for the US beef population. While all the studies above involve convenience samples, they provide an acceptable estimate of PI prevalence. Similar information for fetuses, baby calves, heifers, and cows is not as available. It has been shown that approximately 81% of fetuses will become persistently infected if their dam is transiently infected during the risk period of 74 and 82 days gestation and it is assumed that this risk period extends to 120 days, which is the onset of fetal immunity (Stokstad and Loken, 2002; Stokstad et al., 2003). For the birth of PI calves, then, transient infection must occur during the risk period, so information on the incidence of TIs in at-risk dams would give an indication of the fetal and calf prevalence. The incidence of transient infections in all cattle is unknown, however, as is the incidence during the risk period. Logically, fetal prevalence of BVDV must be no less than baby calf prevalence, and is likely somewhat lower due to the higher mortality rates in PI calves. Calf prevalence has been estimated, but with questionable results due to loss to follow-up (Wittum et al., 2001; Cleveland, 2003); only 72% of calves were available for retesting, and it is possible that some of the mortalities in the calves lost to follow up were PI and would have raised the prevalence estimate. The single estimate of heifer prevalence, likewise, was marred by excessive loss to follow-up and, more importantly, a lack of external validity

(Cleveland, 2003). Only one herd, with three management groups, was tested, so the heifer prevalence has little bearing on the national population. Adult cow prevalence estimates are limited to a single research report, stating a failure to detect any PIs in a sample of 1000 (White et al., 2007). Determination of the proportions of PI fetuses, calves, cows and heifers within the beef industry is important to production of an accurate assessment of risk, as these comprise a significant portion of the imports to cow-calf herds.

A study of 2,000 auction market-derived yearling steers in one feedlot found a 0.3% prevalence, with a 95% confidence interval (CI) of 0.14-0.65% (Loneragen et al., 2005). A smaller study, of 938 feeder calves entering two stocker operations, found a prevalence of 0.33% with a 95% CI of 0.299-0.360% (Larson et al., 2005). However, diagnosis in both studies relied on the specificity of skin biopsy IHC to PI animals, which has a questionable specificity to persistent infections (see below, under Testing), and may have overestimated the prevalence. Fulton et al. examined over 21,000 500-pound calves entering a Kansas feedlot from order buyers in several southern and southeastern states and found a PI prevalence of 0.40% (Fulton et al., 2006). However, the follow-up test was performed after only 48 hours, and viremia has been noted in transiently infected animals for more than 10 days (Patel et al., 2002). The animals in the study by Fulton et al. may have become TI during transit, so the prevalence figure may be overestimated. In 1,045 feedlot calves, part of a group of 5,129 seven to ten month old calves from a variety of auction markets in Western Canada, only one was viremic, and it was lost to follow-up before being diagnosed as persistently infected (Taylor et al., 1995). The occurrence of mucosal disease in the unsampled calves led the authors to infer a prevalence between 0 and 0.1%.

A study of young purebred bulls in Kansas, using samples from all bulls offered for public or private sale in one year, found a BVDV prevalence of 0.67%, with 95% CI of 0.35-0.99% (Gnad et al., 2005), based on 2,520 serum samples. An immunoperoxidase monolayer microtiter assay was used for testing, which has no established sensitivity or specificity on serum samples in the published literature. The study only included breeding bulls sold in the state of Kansas, but all animals <2 years of age sold during the year 2001 were included. No follow-up was performed, so some of the animals may have been TI and this would bias the results to a higher prevalence estimate.

Beef herd prevalence overall has been estimated to be 3.75%, with a 95% CI of 0.26%-7.24% (Wittum et al., 1997) in 80 randomly selected herds with at least 20 breeding females. Serum was collected from all calves in the herds and an immunoperoxidase monolayer microtiter assay was used for testing, although the sensitivity of this test has not been validated in the published literature. The study focused on commercial beef herds and used a geographically diverse sample. Based on 76 randomly selected herds from the same study (Wittum et al., 2001), herd prevalence was estimated at 3.95% with a 95% CI of 0.27%-7.62%. No reason was given for excluding 4 herds previously included, but it appears they were reclassified as suspect herds, rather than randomly selected. In BVDV suspect herds, the estimate is higher, with herd prevalence estimates ranging from 10%,(10/100 herds), in diagnostic laboratory submissions in Ontario (Alves et al., 1996), to as high as 19.2% or 10 in 52 herds (Wittum et al., 2001). Risk of virus introduction, therefore, is higher from herds showing signs of BVDV infection.

### *Testing*

Testing imported cattle, including calves of pregnant imports is one method of decreasing risk of BVD introduction. Numerous tests are available for detection of virus, including virus isolation, antigen capture ELISA, IHC and PCR for detection of BVDV (Table 1).

**Table 1.1: Tests currently available for BVDV**

<b>Test</b>	<b>Cost</b>	<b>Sensitivity</b>	<b>Pool Sensitivity</b>	<b>Sample types possible</b>
VI	Moderate to high	0.64-1.0	N/A	Whole blood, serum, tissue
IHC	Low	0.97-1.0	N/A	Whole blood, tissue
ELISA	Low	0.47-1.0	N/A	Whole blood, serum, tissue
PCR	Moderate to high	0.9-1.0	1.0 (Kennedy et al., 2006)	Whole blood, serum, tissue supernatant
Microplate VI	Moderate	0.5-0.85	N/A	Serum

Virus isolation (VI) is considered the gold standard by most studies of other tests (Frey et al., 1991; Mignon et al., 1992; Deregt and Prins, 1998; Ozkul et al., 2002; Cornish et al., 2005; Walz et al., 2005)). As such, few studies discuss its sensitivity or specificity. The few studies found in this review that did examine the effectiveness of VI were all of similar quality. Older studies tend to report high specificity with mediocre sensitivity of 83% on 1006 buffy coat samples from 989 6-month old steers (Mignon et al., 1992) and 83% on liver and kidney tissue from 105 aborted fetuses (Ellis et al., 1995). More recent studies, however, with improved techniques, report higher sensitivities on buffy coat samples, with correct identification of 41 of 45 samples (91%) previously established as BVDV-positive and 15 of 15 negative controls (Ozkul et al., 2002). In an unpublished study, VI detected 30 of 41 calves (73%) identified as PI by one of five diagnostic tests with 100% specificity on 782 samples negative by all five tests (Walz et al., 2005).

Microplate virus isolation, as a cheaper and faster method, has been applied to some extent on serum. One study on serum, buffy coat, and tissue homogeonate found 85% sensitivity and 100% specificity when compared to VI, although data on the sensitivity to different samples was not made available (Saliki et al., 1997). An unpublished study found only 50% sensitivity on serum, however, with 100% specificity (Walz et al., 2005). Further investigation is indicated.

Opinions as to the usefulness of ELISA tests vary by sample type and report. One study examined the performace of four different ELISA tests on buffy coat samples compared to full plate virus isolation. Each test exhibited 100% specificity. Three of the tests were highly sensitive (range 94-97%) but one test was only 64% sensitive (Brinkhof et al., 1996). No explanation was given for the low sensitivity of the one test. Two older studies found 100% sensitivity and specificity (Mignon et al., 1992) and 97% sensitivity and 99% specificity (Sandvik and Krogsrud, 1995) on buffy coat samples. Another study compared the sensitivity and specificity of ELISA to BVDV on supernatent from skin samples with VI results on tissue and buffy coat samples from the same animals, finding 100% sensitivity and 98% specificity (Cornish et al., 2005), but the samples included were entirely from viremic animals, with a few TI animals and mostly PI animals. A study including both confirmed PI animals and field samples from certified BVDV-free herds found 100% sensitivity and 99.6% specificity (Kuhne et al., 2005) and gives more confidence in the specificity estimate, as a greater variety of non-PI



animals was included. Serum samples, by comparison, seem to have a low sensitivity when compared to VI. Graham et al. (Graham et al., 1998a) estimated 46-48% sensitivity and specificity of 95% in 214 field samples from BVDV-suspect animals. Saliki et al. (Saliki et al., 1997) found 85% sensitivity with 100% specificity with 224 field samples and 30 titrated virus samples. In these two studies of ELISA on serum samples, the gold standard was held to be VI, which has a low sensitivity with serum samples, as noted above. The estimated ELISA sensitivity may be too high, in consequence, and the specificity may be too low, as some of the samples may have been misclassified by the VI. Another study, however, found sensitivity and specificity of 100% on serum samples from 30 PIs and 30 negative controls previously established by VI (Plavsic and Prodafikas, 2001), which provided a more appropriate measure of infection status based on multiple test results.

Rt-PCR is frequently used on several types of samples. Early studies found that PCR was able to identify multiple strains in both serum and tissue homogeonates (Belak and Ballagi-Pordany, 1991), with sensitivity that may exceed that of VI (Urano et al., 1998). Newer studies have found 100% sensitivity on whole blood (Deregt et al., 2002) and 93% sensitivity on tissue homogeonates (Schmitt et al., 1994) when compared with VI.

PCR is also available for pooled samples of serum or tissue supernatent. Studies have found PCR capable of detecting BVDV in pools of 50 (Braun et al., 2000) to 100 (Weinstock et al., 2001) serum samples, although in each case only one pool was examined. A recent study of 100 positive pools found that PCR had a 95% sensitivity on pools of 30 (Smith et al., 2007). In addition, PCR on pools of 12 to 100 tissue supernatent samples was found to have 100% sensitivity and 97.4% specificity when compared with ELISA results on individual samples (Kennedy, 2006).

IHC is possible on various tissues, but is most commonly used on skin biopsies. A recent study found that IHC had 100% sensitivity for detecting PIs on skin biopsies when compared to VI, but that it misdiagnosed several TI animals (Cornish et al., 2005). An earlier study had suggested, with results from a single TI, that IHC would be specific for PIs, for which it had 100% sensitivity when compared to VI (Grooms and Keilen, 2002), but the evidence is weak due to the lack of variation in samples; sampling only one TI severely limits the external validity of this study. No other studies in the published literature have evaluated this claim further. With

formalin fixed tissues, IHC has shown 98% sensitivity to PI animals dead of mucosal disease and 100% specificity on animals assumed to be negative (Haines et al., 1992).

Difficulties with testing relate to two main issues. First, some PI animals are seropositive, most often due to maternal antibodies and sometimes due to infection with heterologous strains, and antibodies may interfere with VI and ELISA tests (Edwards et al., 1991). This can decrease test sensitivity, and should be taken into account when testing young calves. Second, variation in viremia in PI cattle has been observed (Brock et al., 1998), which may lead to decreased sensitivity for PI cattle and again decrease the test sensitivity. Animals testing BVDV negative after a previous positive test, therefore, cannot be assumed to be transiently infected. It may be necessary to test animals with discordant results a third time in order to increase sensitivity.

It would be desirable to test the fetus of a pregnant import *in utero*, as fetal prevalence is higher than adult prevalence and the calf of a pregnant animal is likely to be a greater risk than the dam. One study has found that the antibody titer of cows carrying a PI fetus may be significantly higher than that of other pregnant cows (Brownlie et al., 1998), but this may be misleading in recently infected or vaccinated animals not carrying a PI fetus. In further inquiries, different cutpoint in an antibody ELISA found sensitivity ranging from 94-100% for detection of a PI fetus, but specificity only ranged from 39-64% (Lindberg et al., 2001). Another technique, involving collection and testing of fetal fluids, found that VI was capable of detecting viremia in a single fetus, but the process may have caused abortion or premature delivery in 14 of 160 cows (Callan et al., 2002). In summary, the available tests for fetal infection are either nonspecific or possibly dangerous to the fetus. More research in this area would be helpful; at present, testing of fetal imports must take place after birth.

### ***Vaccination***

Prevention of herd BVDV infection after introduction to a herd is dependant on herd immunity to decrease the spread of infection following contact. Increased herd immunity may be achieved by vaccination or natural exposure. The use of either modified live (MLV) or killed vaccines, both of which are commercially available and safe (Stokka and Edwards, 1990; Castrucci et al., 1991a; Said et al., 1996; Cortese et al., 1997), may be effective at preventing both viremia with BVDV in heifers using German vaccines, (Frey et al., 2002) and the effects of

BVDV in feedlot calves (Cravens, 1991). Efficacy can refer to a number of effects, such as decreasing viremia and viral shedding or preventing morbidity, although generally refers to preventing disease. One study found that MLV vaccines prevented all detectable viremia and a majority of nasal shedding in 28 experimentally infected calves in comparison to 11 control calves (Dean and Leyh, 1999). Another study detected a decrease in viremia and clinical signs in 15 experimentally infected calves vaccinated with a subunit vaccine compared to 5 controls (Nobiron et al., 2003). A killed vaccine was found to decrease the duration of viral shedding in 8 calves, although no effect was noted on clinical signs when compared with the 8 control calves (Peters et al., 2004). It should be noticed that these are all laboratory studies, rather than field studies, and may not have external validity. One field study, though, in 2581 feedlot calves, divided between 10 pens on two feedlots, noted that average daily gain was increased and rates of fever were decreased when a multivalent vaccine with MLV BVD was added to the normal vaccination scheme, which was used on 2582 calves in 10 pens on the same two feedlots (Schunicht et al., 2003).

The primary goal of vaccination in herd control programs, however, is preventing fetal infection; endemic production of PI calves increases the duration and cost of a BVDV outbreak. MLV vaccines appear to provide good fetal protection, with some level of cross-protection between strains. Cortese et al. (Cortese et al., 1998a) observed fetal protection in 10 of 12 heifers vaccinated for and exposed to BVDV, but the strain of vaccine and challenge virus were unrecorded. Two of 25 calves born to dams vaccinated with a BVDV1 vaccine were persistently infected after BVDV1 challenge (Dean et al., 2003). One study found that 9 of 19 of pregnant animals experimentally infected were protected from fetal infection with BVDV2 after MLV vaccination (Brock and Cortese, 2001). A recent study found efficacy of 33%, 37%, and 100% in groups of 20 heifers exposed to BVDV2, depending on the vaccine protocol used, with the best protection offered by a combination of type 1 and type 2 vaccines (Ficken et al., 2006b). Another study with similar methods found that all 40 fetuses were protected after vaccination of the dams with a BVDV-1 vaccine, regardless of the type of the challenge virus (Ficken et al., 2006a). Similar results were seen with vaccines containing both BVDV types, with 18 of 18 fetuses protected from BVDV1 and 18 of 19 fetuses protected from BVDV2 in one study (Fairbanks et al., 2004) and 10 of 11 fetuses protected from BVDV1 and 10 of 10 fetuses protected from BVDV2 in another (Kovacs et al., 2003). Eleven pregnant heifers vaccinated

with a BVDV-1 virus gave birth to non-PI calves after exposure to PI animals in another study (Patel et al., 2002), indicating protection from a field-style infection. Killed vaccine was found to prevent fetal infection in 15 pregnant cows after experimental infection (Brownlie et al., 1995), though another study observed PI calves from 14 of 24 vaccinated heifers (Zimmer et al., 2002); in neither study was the type of either the vaccine or the challenge virus provided. In all the above studies, an appropriate number of control heifers was observed to give birth to PI calves.

Vaccine duration estimates vary and are based entirely on antibody titers, not viral challenges, although Ficken et al. (Ficken et al., 2006a) found sufficient fetal protection present at 370 days post-vaccination. Cows vaccinated with MLV vaccine had antibody titers detected up to 18 months post-vaccination (Cortese et al., 1998b); the study did not test for antibodies past 18 months. Killed vaccine, however, was noted to produce antibody titers that waned by 12 weeks (Graham et al., 2003). Other cows vaccinated with a killed vaccine in late gestation produced colostrum that gave calves an 8 week antibody half life (Barringer and Rosenberg, 1995). In calves, it is possible that vaccination at weaning increases the half-life of passive immunity (Fulton et al., 2004). It is, at the least, apparent that passive immunity does not interfere with vaccination after 5 weeks of age, as calves receiving BVDV vaccine at 5 weeks of age showed fewer symptoms of BVD infection, compared to control calves, with or without colostrum protection (Zimmerman et al., 2006).

Vaccination coverage is desired to protect the entire herd from viral transmission. Adequate protection against BVD outbreaks is thought to occur with a vaccination coverage of 85-99% in dairies (Cherry et al., 1998), but similar calculations, which rely on animal density and mixing, are unavailable for cow-calf farms. With a lower stocking density, cow-calf farms may have a lower rate of infectious contacts, but the increased mixing between adults and calves could increase the number of infectious contacts and, thereby, the necessary level of vaccine coverage.

### ***Transmission***

Observational data on transmission of BVD within cow-calf herds is not available. Experimental data on transmission suggests that transmission may occur through environmental contamination. Calves housed in a pen contaminated by the birth of PI calves seroconverted,

with and without the presence of the dam of the PI calf (Lindberg et al., 2004). Four calves in the same building as, but with no direct contact with, a PI calf were found to seroconvert, with maximum distance between pens of 10 meters, which suggests airborne transmission of BVDV (Niskanen and Lindberg, 2003). The same study found that a pen housing a PI calf was infective to a susceptible calf entering the day it was vacated, but not 4 days later. However, this work was carried out in a lab setting, so the application of this to US cow-calf production systems (or any real-world production setting) is problematic. It is likely that transmission occurs primarily between PI animals and in-contact susceptible animals, but no studies have defined this relationship in field situations. Most work on BVDV transmission has been carried out with the use of computer models.

The dynamics of BVDV transmission have been modeled in a number of ways for dairy farms. One transmission equation has been suggested by Innocent,  $I=S(1-(1-\epsilon)^{\Pi})$ , where I is infected animals, S is susceptible animals,  $\Pi$  is persistently infected animals, and  $\epsilon$  is the transmission rate, best represented by  $R_0/(N-1)$  (Cleveland, 2003).  $R_0$ , again, is the basic reproduction ratio and N is the number of animals in the group. This model followed the spread of BVDV on a dairy farm after introduction of a single PI adult until a steady-state disease endemicity was reached. The prevalence of PI animals in the herd at the endemic level resembled field data when  $\epsilon$  was set to 0.3 per PI per month (Innocent et al., 1997a). When compared with the results of a mass-action model, the level of agreement validated the assumptions of the model (Innocent et al., 1997b), but this does not equate with validation using field data. The results of this model are not likely to be of use on cow-calf farms, due to the difference in management techniques.

Transmission of BVDV within a cow-calf herd has been modeled using equations adapted from Innocent et al. (Innocent et al., 1997a) for use in cow-calf herds (Cleveland, 2003). Seroprevalence and PI prevalence data from three herds were used to validate the model under field situations of low PI prevalence. The model was most sensitive to the parameter of infectious contact rate, referred to here as  $R_0$ . Agreement with field data was highest when  $R_0$  was set to 7, as opposed to 3 or 10. With a modeled population of 400, this is equivalent to an  $\epsilon$  of 0.0175, compared to 0.0075 or 0.025. This is much lower than Innocent et al. suggested, but may be appropriate given the lower density of the cow-calf farm in comparison to a dairy. The transmission rate was chosen based on unpublished serologic field data, and the number of PIs

present in the herd in the second year after BVDV identification was not significantly different from that predicted by the model. However, these results were also not significantly different from those predicted with an  $R_0$  of 3 or 10. Thus, the  $R_0$  of BVDV in cow-calf herds is likely to fall between 3 and 10, but further speculation is not possible with this model.

Mathematical models of BVDV in dairy herds also have found that the parameter representing transmission from PI calves to other groups was the most influential on the size and impact of an epidemic (Ezanno et al., 2007). This underscores the necessity of defining the possible range or distribution of  $R_0$  for cow-calf herds in order to properly model the disease.  $R_0$  values for dairy herds have been suggested to range from 2.3 to 35 (Cherry et al., 1998), with the lower value indicating transmission from TI animals and the higher representing infectious contacts for PI animals. The value for PI animals is an estimate and is based on the high-density dairy herd, and so would not be appropriate for cow-calf models. Cherry et al. set the  $R_0$  of 2.3 for TI animals based on unpublished data, but similar models have thrown doubt on the role played by TI animals in BVDV transmission, and the issue is controversial. One model found that including transmission by TIs resulted in a similar steady state, arrived at one year earlier (Innocent et al., 1997a). Another found that increasing the transmission rate for TIs 20-fold did not significantly change the persistence of herd infection (Viet et al., 2004a). Assuming lifelong immunity after transient infection, Cleveland found that including infection from TIs would significantly decrease the clearance time for the herd; (Cleveland, 2003) however, that might become insignificant if waning immunity was included in the model. If TI transmission is modeled, however, all of the above studies agree that the  $R_0$  for TI animals is much lower than the  $R_0$  for PIs.

The range in estimates of  $R_0$ , for PIs or TIs, reflects both uncertainty regarding the true values and variation in the value based on other factors, including management factors and viral strain, so the effective reproductive rate likely varies between herds. A herd outbreak investigation by Taylor et al. suggests that transmission rates are affected by management factors, such as stocking density and maintaining separate groups (Taylor et al., 1994). In that study one herd with three management groups was followed for 8 months after a BVDV outbreak, and lower rates of persistent infection were observed in the group with a lower stocking density and less confinement (Taylor et al., 1994). Viremia levels in infected animals are one more potential variable in the  $R_0$ , as higher viremias may cause higher shedding rates.

Transiently infected calves in a research setting have shown significantly different viremia levels with different BVDV strains, so these strains may show different  $R_0$  values (Walz et al., 2001). These natural variations indicate that a range of values would be the best way to model  $R_0$ , which could be influenced by a number of issues in the field.

Transmission within a herd is related not only to the rate of transmission from introduced PIs, but also the rate at which PIs are produced endemically. The endemic PIs will maintain the infection within the herd by sustaining the infection of susceptible animals after the death of the imported PIs. The time at which the virus is introduced is key to production of PI calves (Sprecher et al., 1991; McGowan et al., 1993b; Woodard, 1994). The fetus is only susceptible to persistent infection between 40 and 125 days gestation (Stokstad and Loken, 2002). In cow-calf farms, this period spans from early in the breeding season until weaning. Limiting risk factor exposure during this period curbs production of PI calves within the herd, allowing faster clearance of infection.

### ***Impact***

Infection with BVDV primarily affects calf health and herd reproduction. Persistently infected animals are assumed to have higher mortality and morbidity than non-PI animals, although reliable estimates of these rate are not available for calves pre-weaning. Houe found that PI animals were removed from dairy herds at a higher rate than non-PI animals, but his data included culling with mortality and only 3 PI calves of <3 months were in the population of the study (Houe, 1993). A case study of a Saskatchewan cow-calf herd followed calf deaths for one year and recorded mortality due to presumed persistent infection, but half of the calves dying in the pre-weaning period were not tested for BVDV (Taylor et al., 1997). A study of PI prevalence in 133 geographically diverse beef herds found that 10 of 56 calves positive for BVDV at initial testing died pre-weaning, but no follow-up was used to establish their status as persistently or transiently infected and 3 additional calves were lost to follow-up (Wittum et al., 2001); it may be inferred that a majority were PI, as 33 of 43 retested calves found to be positive. A comparison of congenitally infected calves (calves with a BVDV antibody titer at birth) and persistently infected calves with non-infected calves in two dairy herds found an increased morbidity and mortality rate among infected calves pre-weaning, but did not distinguish between congenitally infected and PI calves (Munoz-Zanzi et al., 2003). Experimentally produced PI

calves were all noted to be poor doers in one study, but no normal calves were observed for comparison and the study was carried out in a laboratory setting limiting external validity (Stokstad and Loken, 2002). From these studies, it is likely that PI calves will experience higher morbidity and mortality rates than TI calves. However, due to the low prevalence of PI animals (see above), it is also likely that the cost of PI morbidity and mortality is outweighed by the cost of TI morbidity and mortality in calves infected by the PI animals in the herd.

The BVD virus has several effects during transient infection, including immunosuppression, which is one of the main causes of TI mortality. Immunosuppression increases both susceptibility to and risk of dying from another pathogen. Haines et al. (2004) found an increased prevalence of BVDV in calves dying of myocarditis, most associated in this study with *Haemophilus somnus*, and pneumonia, associated with *Mannheimia hemolytica*, *Mycoplasma bovis*, and *H. somnus*, compared to calves dying of non-infectious causes. Infection status for BVD was established by IHC, so it is uncertain whether the calves were transiently or persistently infected (Haines et al., 2004). In another study where BVD infection was established by VI, TI calf mortality was observed to be 11% in two cow-calf herds with a BVDV outbreak (Campen et al., 2000). An older case report noted a 23% mortality rate in calves during a BVDV outbreak in an Australian herd (Kirkland et al., 1990). However, these three mortality estimates are based on case reports of limited numbers of farms. There are no published observational studies or clinical trials of mortality due to transient BVDV infection on a larger scale.

Morbidity in TI animals is also a significant factor in the cost of BVDV infection in cow-calf herds. General disease symptoms identified for TI animals include weak calves (Woodard, 1994), diarrhea (Klingenberg et al., 1999), and undifferentiated respiratory disease (O'Connor et al., 2001). Calf morbidity rates were estimated at 26.7% during a BVDV outbreak in a single Argentine farm, but the diagnosis of BVD was made based only on necropsy of 6 calves, so the morbidity rate may be overestimated (Vottero et al., 1992). No other estimates of morbidity rates are available in the published literature. The specific clinical signs and risk of morbidity depend in part on the strain involved. Comparing susceptible calves inoculated with two laboratory-isolated field strains, Bolin et al. (Bolin and Ridpath, 1992) noted an increased morbidity and mortality with one strain. Castrucci et al. (Castrucci et al., 1991b) also noted an increase in morbidity and mortality in calves inoculated with cytopathic rather than non-



cytopathic virus. Kelling et al. compared five different strains isolated from aborted fetuses and also found that some strains induced more severe symptoms and higher morbidity rates in experimentally inoculated 6 to 9 month old calves (Kelling et al., 2002).

One of the additional costs associated with BVDV in cow-calf farms comes from the side effects of morbidity due to BVDV infection. Calves tend to experience a weight gain depression due to general morbidity. Wittum et al. found that calves with morbidity due to undifferentiated causes experience, on average, a 15.9 kg decrease in weaning weight (Wittum et al., 1994). Martin et al. (1999) found that seroconversion to BVDV was associated with lower weight gains in feedlot calves, and hypothesized this was most likely through its association with respiratory disease; this could be extrapolated to pre-weaning calves, as the biological basis for the effect would not be changed by age, although the risk of respiratory disease would.

Morbidity in BVD outbreaks is also due to the potentiation of other pathogens. An experimental study noted that calves co-infected with BVDV and *Mannheimia haemolytica* experienced more severe morbidity than calves infected with only one of the agents or control calves (Ganheim et al., 2003). Another laboratory study found that simultaneous infection with BVDV and Infectious Bovine Rhinotracheitis virus (IBRV) produced more severe clinical signs than infection with IBRV alone (Castrucci et al., 1992). These results suggest some amount of synergy between the BVDV and other pathogens. In a large study of seroconversion in field outbreaks, the majority of BVDV seroconversions were associated with other viral infections, indicating that BVDV may be more of a potentiating factor than a primary disease pathogen (Graham et al., 1998b). Bjorkman et al. noted a positive association between BVDV and *Neospora caninum* serostatus in Swedish dairy cattle experiencing abortions (Bjorkman et al., 2000). As noted above, a study of feedlot deaths found that BVDV seroconversion was significantly associated with deaths due to *Hemophilus somnus*, *Mycoplasma bovis*, and *Mannheimia hemolytica* (Haines et al., 2001). Martin et al. found that seroconversion to BVDV led to an increase in respiratory disease risk in feedlot calves (Martin et al., 1999), so it is possible that this effect is present in pre-weaning calves.

Infection with BVDV can affect farm reproduction parameters, including conception rate and abortion rate. Experimentally, heifers first infected with BVDV within a week of insemination were found to have significantly lower conception rates than control heifers (McGowan et al., 1993a). When the study was expanded to field conditions, the same

relationship held for heifers, though not for cows (McGowan et al., 1993b). This effect is notable at the herd level, as well. In one study, four of 8 dairy herds experienced a significant decrease in conception rate when the oldest PI animal was conceived, which is assumed to be the time of initial BVDV infection (Houe and Meyling, 1991b). In a study of a single dairy herd following initial BVDV isolation in a calf, and utilizing historical records from the previous year for a control, a significantly lower conception rate and a significantly higher proportion of early abortions and retained placentas was noted (Larsson et al., 1994). Management changes between the control period and the case period are not reported, but could have been responsible for the change in the conception rate. In a large cross sectional study, cow-calf herds with PIs present in the herd had 5% lower pregnancy rates than herds without PIs (Wittum et al., 2001). A large, multi-year case-control study of the reproductive effects of BVDV in Norwegian dairy herds found a 3% higher abortion rate in herds with endemic BVDV as determined by antibody levels in bulk milk samples (Fredriksen et al., 1998). A study of seroprevalence to BVDV and history of recent abortions in dairy cows found a significantly lower abortion rate in seropositive cows (Hassig and Lubsen, 1998), but the study did not have data regarding the time of seroconversion, so only a single sample was taken and seroconversion was not assessed so the seropositive cows may have been protected from BVDV by immunity from a previous infection.

## **Conclusion**

This literature review identified several knowledge gaps for further research to fill. The prevalence of PI animals in adult beef cattle is likely low and therefore of little importance, but this popular belief should be confirmed. The PI prevalence in fetuses and baby calves is also unknown, however, and is an important parameter for risk calculations. The amount of morbidity and mortality among PI calves is undefined, as well, and could impact the transmission of virus within a herd; the uncertainty about the TI morbidity and mortality rates, in contrast, will have a greater impact on economic analysis of BVDV risk, assuming TIs outnumber PIs. Transmission dynamics of BVDV on cow-calf farms are currently a matter of conjecture; while difficult to establish experimentally, model creation and validation may enable confirmation or refutation of currently held beliefs. The level of risk posed by neighboring herds, especially from fence-line contact, is also in question. There are some experimental ways that these could be addressed.

This literature review, outlines the basic structure of BVDV introduction and spread on cow-calf farms. The most important source of BVDV infection is contact with PI animals. Good biosecurity protocols, especially testing, decrease this risk. Importing from tested known negative herds would decrease the risk posed by imported animals. Vaccination may prevent spread of virus once introduced and mitigate its impact, which includes reproductive effects in adults and calf morbidity and mortality. The high cost of BVD necessitates good control and prevention strategies; a risk analysis model incorporating the identified risks can be a valuable tool in preparing those strategies.

## **CHAPTER 2 - Sensitivity of PCR for detection of bovine viral diarrhea virus in pooled serum samples and use of pooled PCR to determine prevalence of bovine viral diarrhea virus in auction market cattle**

### **Abstract**

Two reverse transcription-nested PCR tests, one quantitative (QRT-nPCR) and one standard (RT-nPCR), were evaluated to assess sensitivity for detection of bovine viral diarrhea virus of a single positive serum sample in a pool of 30. The RT-nPCR and the QRT-nPCR each detected 95 of 100 positive pools. There was no observable association of sensitivity with genotype 1 or 2 for either test. The RT-nPCR was chosen for use in the field prevalence portion of the study to estimate the prevalence of BVDV in adult beef cows. Serum samples were obtained from USDA brucellosis testing labs in three Midwestern states. Samples originated from auction markets and private treaty sales throughout the three states. A total of 2990 serum samples were collected and randomly pooled into 100 pools for testing. The standard RT-nPCR was applied to pools to determine the prevalence of BVDV in adult cows. Two of the 100 pools were positive, and each positive pool had a single positive individual sample upon confirmation. The estimate of apparent BVDV prevalence in adult cows in this study was 0.07%, with a 95% confidence interval of 0.01% to 0.24%.

### **Introduction**

Bovine viral diarrhea virus (BVDV) is a common disease of US cattle herds (Paisley et al., 1996). It causes a range of effects, including immunosuppression leading to increased disease incidence (Kozasaa et al., 2005) and reproductive disorders such as abortion (Fredriksen et al., 1998), decreased conception rate (Larsson et al., 1994), early embryonic death (McGowan et al., 1993a), and congenital infections (Munoz-Zanzi et al., 2003). The disease costs producers and the livestock industry through increased treatment expenses, calf and pregnancy loss, and decreased weight gain (Larson et al., 2005).

BVDV is spread primarily through persistently infected animals (PIs) (Houe, 1999). As such, these animals are the most important target of control and biosecurity programs. Control programs based on herd testing protocols have been predicted to be cost-effective based on disease models (Stott et al., 2003). In dairy herds, most control programs rely to some extent on bulk tank milk sampling, which is a low-cost herd screening method (Mars and Van Maanen, 2005), but this is not available for beef herds. Instead, beef herds must rely on samples from individual animals, such as blood or tissue samples. Testing costs may be decreased by pooling samples; for calves entering feedlots, limited data indicates that pooling serum samples for PCR followed by confirmation of individuals in positive pools may be more cost-effective than individual testing on all samples (Larson et al., 2005).

The advent of polymerase chain reaction (PCR) tests for BVDV has enabled sample pooling, due to the high analytic sensitivity of PCR. Additionally, PCR tests are not as easily confounded by antibodies as ELISA and virus isolation tests may be in some circumstances (Zimmer et al., 2004). These characteristics of PCR testing may make it more effective for pooling serum samples for testing. However, the cost-effectiveness of any pooling strategy depends on both the sensitivity of the test and the prevalence of the disease in the population. While the sensitivity of PCR on pooled sera has not been determined, studies have detected one positive serum sample in a pool of 50 and 100 samples (Weinstock et al., 2001). Using pooled supernatants from ear notch samples, Kennedy et al. detected one positive animal among 99 negatives with good sensitivity and specificity (Kennedy et al., 2006). A stochastic model accounting for dilution effects of pooling calculated that sensitivity would decrease by 6-7% for pools of 20 compared to individual testing (Munoz-Zanzi et al., 2006), but no laboratory-based validation of pooled serum PCR sensitivity has been performed.

Knowledge of PI prevalence is also vital to determining the risk posed by importation of cattle and determining the cost effectiveness of pooling programs. Prevalence of PIs in the US beef herd is known to some extent for calves (Loneragen et al., 2005), heifers (Cleveland et al., 2004), and bulls (Gnad et al., 2005). Little data are available on the prevalence in adult cows, however, one study found no PIs in 1000 non-pregnant adult cattle that were purchased from multiple operations (White et al., 2007). Wittum et al. traced back the dams of 45 of 56 PI calves detected in a survey of 19,000 calves and found that 3 were PI (Wittum et al., 2001);

assuming that PI cows will produce always PI calves (Houe, 1995b) and that test sensitivity is perfect, adult prevalence could be roughly estimated at 0.02% in those 45 cows.

The purpose of this study is to estimate the sensitivity of PCR for a single BVDV-positive serum sample in a pool of 30 serum samples and to use the pooled PCR to determine the prevalence of BVDV infection in adult beef cattle sold through Midwestern auction markets and private treaty sales.

## **Materials and Methods**

### ***Pooled PCR sensitivity***

#### ***Samples***

Sample size was determined to establish a 95% confidence interval for test sensitivity with a confidence width of  $\pm 5\%$ . One hundred BVDV-positive serum samples were obtained from diagnostic and research samples from veterinary labs in New York, Kansas, and Alabama. Samples had previously been diagnosed as BVDV-positive by a variety of tests at the originating laboratory. The BVDV-negative samples that were incorporated into the pools were obtained from 782 beef calves that were BVDV-negative by skin biopsy immunohistochemistry, whole blood virus isolation, and serum virus isolation.

#### ***Testing***

Two PCR protocols were used throughout the validation phase of the project, a reverse-transcription-nested PCR (RT-nPCR), and a quantitative RT-nPCR (QRT-nPCR). Initially all individual positive samples underwent confirmation testing by each of the two PCR tests in duplicate. The QRT-nPCR established the genotype (BVDV 1 or BVDV 2) of virus in the sample. For evaluation of test sensitivity to pools, pools were formed with 100  $\mu$ l of a single known-BVDV-positive sample and 100  $\mu$ l each of 29 known-BVDV-negative serum samples. Pool size was chosen to provide sufficient pool size for economy, based on the presumed prevalence of BVDV in adult cattle, without excessive dilution. Pools were tested in duplicate with both the RT-nPCR and the QRT-nPCR.

#### ***PCR***

The reverse transcription nested PCR (RT-nPCR) has been previously described in detail (Givens et al., 2001). Briefly, RNA was isolated from samples using the QIAamp® viral RNA mini kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. All steps of the RT-nPCR were performed in a single-tube reaction. In the first round, the outer primers, BVD 100 (5'-GCTAGCCATGCCCTTAG-3') and HCV 368 (5'-CCATGTGCCATGTACAG-3') amplified a 290 base pair sequence of the 5' untranslated region of the viral genome. In the second round of the reaction, the inner primers BVD 180 (5'-CCTGAGTACAGGGDAGTCGTCA-3') and HCV 368 amplified a 213 base pair sequence within the first amplicon. After completion of the PCR cycle, 5 µl of the RT-nPCR products were separated by 1.5% agarose gel electrophoresis. Ethidium bromide staining allowed visualization of the RT-nPCR using an ultraviolet transilluminator.

In addition, QRT-nPCR was performed to detect BVDV, allowing simultaneous detection and differentiation of BVDV 1 and BVDV 2. A detailed description of quantitative PCR has previously been published (DeGraves et al., 2006). Briefly, RNA was isolated from samples as described above. For the first round of the nested reaction, a Lightcycler® RNA Amplification Kit HybProbe<sup>a</sup> was used. The outer primers, BVD 180 and HCV 368 amplified a 213 base pair PCR product from 5 µl of RNA sample. For the second round of the quantitative nPCR a LightCycler® FastStart DNA Master HybProbe Kit<sup>a</sup> was used. The PCR primers for this reaction were BVDVL1 (TGCCATGTACAGCAGAGATTT) and BVDVU3 (CATGCCCAAAGCACATCTTA). The hybridization probes used in this reaction were a BVDVs1dg probe (AYGRAYACAGCCTGATAGGGTGY) labeled on the 3' end with 6-carboxyfluorescein, a BVDVdg2 probe (CAGAGACCTGCTATTCCGCTAGTAAA) labeled on the 5' end with Cy5.5 and containing a 3' phosphate to prevent elongation, and a BVDs2 probe (CAGAGGCCCACTGTATTGCTACTAAA) labeled on the 5' end with an Alexa Fluor 647 and containing a 3' phosphate to prevent elongation (Studer et al., 2002). After completion of both rounds in the Lightcycler® instrument<sup>a</sup>, data were analyzed and displayed as 670:530 nm fluorescence ratios for BVDV 1 and 705:530 fluorescence ratios for BVDV 2.

### ***Adult Cow Prevalence***

The USDA Brucellosis testing laboratories in 3 Midwestern states provided serum samples collected from adult cows at auction markets and private treaty sales. Following

*Brucella* testing, personnel at Brucellosis testing labs selected and shipped the frozen serum samples to Kansas State University, where they were cataloged and stored at -80°C. Sample size was calculated to detect a BVDV prevalence of 0.1% with 95% confidence.

The RT-nPCR test was chosen for use in the field prevalence portion of the study as it is the standard test and easier to perform. Individual samples were pooled in groups of 27 to 30 and tested for BVDV using RT-nPCR, as described above. Individual samples from positive pools were tested by serum virus isolation (Givens et al., 2003a) and, if necessary, by RT-nPCR to identify the number of individual positives.

### ***Data analysis***

Sensitivity and prevalence estimates, with confidence intervals were calculated with the exact methods of proc freq in SAS<sup>b</sup>; measures of association were calculated with proc genmod in SAS<sup>b</sup>.

## **Results**

### ***Pooled PCR Validation***

#### ***Individual Samples***

Of the individual samples, 60 were BVDV 1 and 36 were BVDV 2 by QRT-nPCR. Of the four remaining isolates, two were negative by the QRT-nPCR assays and therefore not typed, and the genotype could not be determined for the other two because the QRT-nPCR was positive for both type 1 and type 2. The RT-nPCR detected 97/100 individual samples; two false negatives were BVDV type 1 and one was not typed. The QRT-nPCR detected 98/100 individual samples; the two false negatives were not typed. Only one sample, which was not typed, was not detected by either the QRT-nPCR or the RT-nPCR. There was no association observed between BVDV genotype and sensitivity of RT-nPCR ( $p=0.06$ ) on individual samples. There was a significant trend for QRT-nPCR not to detect the untyped samples ( $p<0.05$ ), but no association between the sensitivity of QRT-nPCR and BVDV 1 ( $p=0.08$ ) or BVDV 2 ( $p=0.10$ ).

#### ***Pooled Samples***

The RT-nPCR detected BVDV in 95/100 pools. Three of the false negatives were also negative with individual RT-nPCR; the two additional false negatives were BVDV type 2.



The QRT-nPCR detected 95/100 pools. Two of the false negatives were also negative with individual QRT-nPCR; two additional false negatives were BVDV 1 and one was BVDV 2. Four pools were negative by both the RT-nPCR and the QRT-nPCR. There was no association observed between BVDV genotype and sensitivity of the RT-nPCR ( $p=0.17$ ) on pooled samples. There was a small significant trend for non-detection of untyped samples by QRT-nPCR ( $p<0.05$ ), but there was no association between QRT-nPCR sensitivity on pools and BVDV genotype 1 ( $p=0.06$ ) or 2 ( $p=0.06$ ).

Sensitivities for individual and pooled samples are shown in Table 2.1. No difference in sensitivity was detected between the individual tests, between the pooled tests or between the individual and pooled test protocols ( $p>0.05$  for all associations). As the two tests were equivalent, the standard RT-nPCR was selected for use in the adult cow prevalence study.

**Table 2.1: Sensitivity of tests used to detect BVDV in samples.**

Test	Sensitivity	Upper 95% Confidence Interval	Lower 95% Confidence Interval
RT-nPCR	0.970	0.995	0.916
QRT-nPCR	0.980	0.998	0.930
Pooled RT-nPCR	0.950	0.985	0.887
Pooled QRT-nPCR	0.950	0.985	0.887

### *Prevalence*

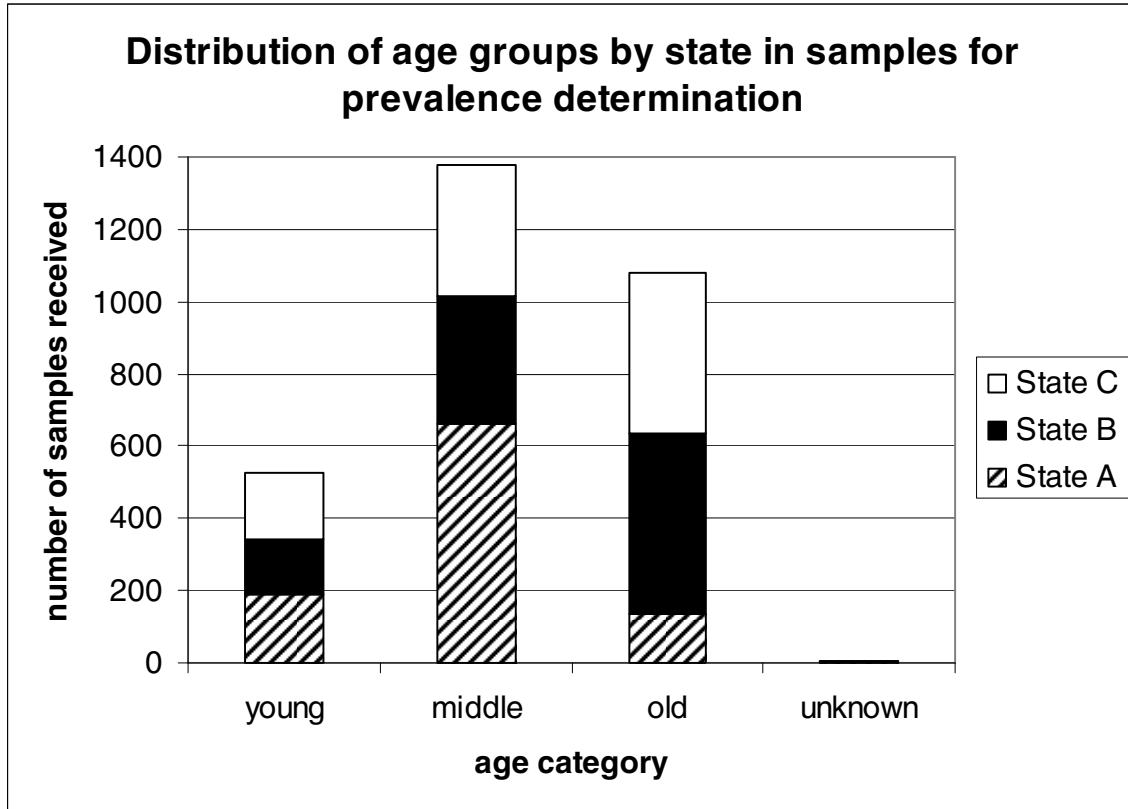
Seven pools did not consist of 30 samples because of the breakage and loss of 7 samples during shipment from one *Brucella* testing lab to Kansas State University and failure of another *Brucella* testing lab to send 3 samples. A total of 2990 serum samples were received from the three states. From state A, 997 samples were collected; 992 were from auction markets and 5 were from private treaty sales. Seventy-two counties were represented and sale dates were from January 7 to March 9 2006. Cow ages ranged from 1 to 11 years. Age was not reported on 4 cows and for 2 cows age was reported as 1+. Average of reported ages was 5 years.

From state B, 1000 samples were collected; 800 were from auction markets and 200 were from private treaty sales. Forty-seven counties were represented and sale dates were from January 2 to January 31, 2006. Reported ages ranged from 2 to 13 years, with 475 listing only the categories of adult (114), short and solid (190), broken-mouth (169), or gummer (2) as ages. Average of reported ages was 5 years.

From state C, 1000 samples were collected from auction market sales, but 7 samples were lost due to broken tubes during shipment, so analysis was performed on 993 samples. Thirty-three counties were represented. Sale dates were not reported but samples were sent to Kansas State immediately after processing and received on April 6, 2006. Reported ages ranged from 2 to 10, with 60 cows reported as 1+, 1 cow reported as 2+, and 338 cows reported as >8 years. Average of reported exact ages was 5.5 years.

Figure 2.1 presents the age distribution of the 2990 samples included by state. The young category includes all cattle aged 1-3, including those reported as 1+ and 2+, the middle aged category is all cattle aged 4-7 or listed as short and solid, and the old category is all cattle aged >8 years or listed as broken-mouth or gummer. Cattle with no reported ages are categorized as unknown age.

**Figure 2.1: Age distribution of samples by state; young=1-3 years (528 total), middle=4-7 years and "short and solid" (1376 total), old=8 years or older and "gummer" or "broken mouth" (528 total); all other animals are grouped as unknown (4 total).**



Pools ranged in size from 27 to 30 samples, with a median of 30 samples and seven pools containing fewer than 30 samples. Two of the 100 pools were positive by RT-nPCR, one containing 30 samples from state B and one containing 30 samples from state C. One individual sample in the pool from state C was positive by microtiter virus isolation. In the positive pool from state B, none of the individual samples were positive by microtiter virus isolation, but one sample was positive by RT-nPCR. Positive samples from both states were obtained from public market sales. The positive sample from state B was from a 6-year-old cow, while the positive sample from state C was from a 5-year-old cow. The estimated prevalence of BVDV in adult cows was 0.07%, with exact 95% confidence intervals of 0.01% to 0.24%.

## Discussion

This study demonstrated a high sensitivity of two PCR assays (RT-nPCR and QRT-nPCR) for detection of BVDV in serum samples, both individually and in pools of 30. The sensitivity of PCR to BVDV in pooled samples reported here relates to a single viremic sample pooled with 29 BVDV-negative samples. Presumably, the sensitivity would increase with the number of positive samples in the pool; however, as the test sensitivities are not significantly different between individual positive samples and positive pools, the increase would most likely be insignificant as well. Given the low prevalence of BVDV observed in the field study, however, the likelihood of more than one positive sample in a pool of 30 is low.

This study does not provide any information regarding pooling with larger numbers of negative samples. The observed decrease in sensitivity related to pooling in this study was less than predicted by previous modeling (Munoz-Zanzi et al., 2006). This difference could be due to the use of positive samples that were previously identified as positives in diagnostic labs. In field samples, sensitivity might be lower due to the greater variation in viremia titer in pre-testing samples, as opposed to samples previously identified by any diagnostic test.

Pooling of serum has been debated for many tests, as antibody interference could decrease sensitivity (Zimmer et al., 2004), but antibodies do not interact with PCR on serum. This study may be considered validation of PCR for detection of BVDV in pools of 30 serum samples. Although BVDV has been detected in larger pools (Weinstock et al., 2001), the optimal pool size depends on the prevalence of disease in the population, the sensitivity of the test at different pool sizes and the cost of the tests used. When prevalence is low, as is the case with BVDV, larger pools become more cost effective if sensitivity is maintained by decreasing the total number of tests required. However, the size of the pool must be weighed against the cost of testing individual samples in positive pools. The cost savings from pooling depend on the number of positive pools and, as a result, the number of individual samples for which further testing is necessary. If prevalence is high, a large number of pools may test positive and the cost of individual testing on positive pools soon becomes prohibitive. At high prevalence, smaller pools or individual testing are more cost-effective. Larger pools may be used with multiple poolings, splitting positive pools into smaller pools before individual testing. However, pool size can be increased to the point of significant dilution, lowering the sensitivity until the number of false negatives nullifies the cost savings of pooling and the value of testing. Pool size decisions should therefore be made on the basis of the test sensitivity on pooled samples and the

prevalence of the disease. With the low prevalence of BVDV noted in this study and others, smaller pools may be inefficient, while larger pools, if the sensitivity has been validated, could be most useful especially if any positive pools are split and retested as smaller pools before individual confirmation. The lowest cost pooling method may be determined more accurately now, using the calculated sensitivity in pools of 30, and Monte Carlo simulations as previously published (Munoz-Zanzi et al., 2000).

No associations between BVDV type 1 or 2 and any test's sensitivity were observed in this study. However, power was low due to the small number of false negatives with any test. QRT-nPCR was significantly less likely to detect samples, individually or pooled, for which no type was known. This is to be expected, however, as the typing in this study was performed by QRT-nPCR; samples that were negative by all QRT-nPCR tests were not typed.

PCR has been recently validated for use on pooled supernatant from tissue samples (Kennedy et al., 2006), but in some instances serum samples may be more available or convenient. In this case we utilized serum samples available in connection with the USDA *Brucella* eradication program. Adult cows moving between farms are often purchased and sold through auction markets. The Midwest region contains a substantial proportion of the beef cows in the US beef cow herd as well as a large number of auction markets. As cows pass through auction markets United States Department of Agriculture (USDA) brucellosis eradication program rules require that a blood sample is collected and tested for *Brucella abortus* antibodies. Private treaty sales of cows are also often tested for *Brucella abortus*. These samples provide a useful cross-section of the adult cows available for purchase.

No previous studies have provided an estimate of prevalence of BVDV in adult beef cows in the US. It is often assumed to be 0.1% based on anecdotal evidence and personal opinion (Munoz-Zanzi et al., 2006; Kennedy, 2006), but no studies have verified that assumption. This study provides an estimate of the prevalence of BVDV in adult auction market cattle in the Midwest US. This prevalence estimate is consistent with previous assumptions that adult cattle are a low-risk source of BVDV introduction to herds, compared with calves and stockers.

Due to the low prevalence of BVDV, this study had insufficient power to detect any associations between prevalence and age. However, the ages of the two positive samples (5 and 6 years) indicate that some middle-aged cows US auction markets are viremic. This is the age range that was sampled most heavily overall, but in states A and B more cattle were sampled

from an older range. The confidence intervals for prevalence in each of the age categories overlap, so we cannot make a claim for an association between age and prevalence.

The results of PCR testing in this study do not differentiate between persistently infected (PI) and transiently infected animals. In order to confirm PI status, an animal must be diagnosed as viremic at more than one time point. The samples used in the sensitivity study were not guaranteed to be from PI animals, and an inability to follow-up precluded identifying the positive animals in the prevalence study as PIs. As PI animals carry the greatest risk of BVDV introduction for a herd (Niskanen et al., 2002b), such follow-up would be very useful in future studies. In a study of calves entering the feedlot, PCR was performed on pools of 5 to 43 whole blood buffy coat samples. It was found to be approximately 90% specific to detection of pools containing PI animals, as opposed to transient infections, when compared with immunohistochemistry (Larson et al., 2005). Validation of the specificity for PI animals of this pooled PCR on serum samples, as opposed to transient viremias, would be valuable. Further as PI animals are persistently viremic and can be detected as positive on any day of their life they are sampled. Transient infections are viremic for only a short period and so offer fewer viremic days to be detected making it more likely that any random sample selected if positive is more likely to be from a PI animal.

The cows sampled for the prevalence estimate do not represent a random sample of all adult beef cows in the US. They are, however, representative of the population of adult beef cows available for purchase in the Midwest. Many of the purchased cattle on cow-calf farms in the Midwest will be sourced from the public market and private treaty sales sampled in this survey. As the primary interest of this study was to identify the risk of importing an adult PI, this was an acceptable sampling strategy. The risk for importing adult PIs from these sales may be higher than the actual prevalence of BVDV in the adult cattle population, as PIs tend to have low fertility and other health issues and are more likely to be culled. In order to define the risk posed by animals at these sales, then, this was the most appropriate sampling strategy.

In conclusion, PCR appears to have good sensitivity for detection of BVDV in pooled serum samples. Prevalence of BVDV in adult beef cattle appears to be very low. This information should be useful in the design and implementation of biosecurity and control programs.

<sup>a</sup> Roche Applied Science, Indianapolis, IN, USA

<sup>b</sup>Copyright (c) 1999 SAS Institute Inc., Cary, NC, USA

## **CHAPTER 3 - A stochastic model to assess the risk of introduction of bovine viral diarrhoea virus to cow-calf herds**

### **Abstract**

A Monte Carlo model was designed to evaluate the introduction of bovine viral diarrhoea virus (BVDV) to cow-calf farms and the effect of different biosecurity strategies. Risks were modeled to include imports to the cow-calf herd and stockers imported to adjacent pastures. The number of persistently infected (PI) animals imported and the probability of BVDV introduction were monitored for three herd sizes, four import profiles, and six testing strategies. Importing stockers and importing pregnant heifers were the biggest risk to introduction of BVDV. Testing stockers decreased the risk they posed, but testing the pregnant heifers was not sufficient to decrease risk unless their calves were also tested. Test sensitivity was less influential than PI prevalence on the likelihood of BVDV introduction, even when all imports were tested. This model predicts the risk of BVDV introduction for individual herd management decisions, and should prove to be a useful tool for cow-calf producers in controlling the risk of BVDV.

### **Introduction**

Bovine viral diarrhoea virus (BVDV) is a common disease of US cattle herds (Houe et al., 1995b; Paisley et al., 1996; Chase et al., 2003). It causes a range of effects, including immunosuppression leading to increased disease incidence (Castrucci et al., 1992; Bjorkman et al., 2000; Kozasaa et al., 2005) and reproductive disorders such as abortion (Fredriksen et al., 1998), decreased conception rate (Houe and Meyling, 1991a; McGowan et al., 1993a; McGowan et al., 1993b; Larsson et al., 1994; Wittum et al., 2001), early embryonic death (McGowan et al., 1993a; McGowan et al., 1993b), and congenital defects (Munoz-Zanzi et al., 2003; Ellsworth et al., 2006). The disease costs producers and the livestock industry through increased treatment expenses, calf and pregnancy loss, and decreased weight gain (Wittum et al., 1994; Gunn et al., 1998; Bennett et al., 1999; Larson et al., 2002).



Infection of a fetus between 40 and 125 days gestation leads to a persistent infection (Stokstad and Loken, 2002). Persistently infected animals (PIs) shed virus for life through oculonasal and other discharges, including feces (Confer et al., 2005) and are generally considered to be the primary source of BVDV introduction to a herd (Houe, 1999; Niskanen et al., 2002b).

Biosecurity against BVDV introduction is limited to testing imported animals and avoiding potentially infectious contact with infected herds. Biosecurity programs based on herd testing protocols have been predicted to be cost-effective based on disease modeling of Scottish cow-calf herds (Stott et al., 2003). A variety of antigen tests have been developed to detect viremic animals, with positive results on samples taken one week apart indicative of a PI. Testing imported animals may decrease the introduction of BVDV. Avoiding the risk factors of herd contact on fencelines and communal pastures (Valle et al., 1999) may also decrease risk of herd infection.

Stochastic models for BVDV spread within farms have been developed and refined for dairy herds (Innocent et al., 1997a; Innocent et al., 1997b; Cherry et al., 1998; Viet et al., 2004a; Viet et al., 2004b; Viet et al., 2005; Viet et al., 2006; Ezanno et al., 2007), and one model exists for spread of BVDV through an endemically infected cow-calf herd (Cleveland, 2003). These models predict the viral spread and disease impact in currently infected herds, but this does not provide useful information in regards to biosecurity for uninfected herds. One partial budget analysis examined the efficacy of testing for BVDV in incoming feedlot calves (Larson et al., 2005), but management differences make the results inapplicable to cow-calf operations. No models for introduction of BVDV to a naïve cow-calf herd are available in the literature. The objective of this study was to develop and apply a stochastic risk analysis model for the introduction of BVDV to a cow-calf herd.

## **Model Building**

### ***1. Model Structure***

The model developed is based on the risk posed to herds by the introduction of persistently infected animals. Introduction of PIs was modeled to come from two sources:

imported animals and fenceline contact with imported stockers, (9-12 month old steer and heifer calves purchased for grazing during the summer). Distributions assigned to the risk level of each of these three sources can be seen in Table 3.1.

The risk of PI introduction by imported animals is modeled specific to age, purpose, and reproductive status of the imported animals. Specific classes of imports modeled include replacement heifers for the breeding herd (either pregnant or open), yearling bulls for use in the breeding herd, stockers and young calves (1-7 days old for grafting onto lactating cows). The prevalence distribution for youngstock is applied to bulls, replacement heifers, and stockers. Typical management practice in US cow-calf herds is to import replacement heifers from a single herd. Therefore, there is potential for clustering of PIs in heifers according to herd prevalence. This is modeled by first assigning a binomial value to the BVDV status of the source herd (based on herd prevalence). The prevalence of heifers in a positive herd is then modeled as the youngstock prevalence divided by the herd prevalence. Persistently infected females are unlikely to produce a non-PI calf, so it is assumed that any pregnant import that is a PI is also carrying a PI fetus (Houe, 1995a).

Stockers are young weaned calves being grazed for weight gain prior to sale to feedlots, and are often purchased through an auction market. Stockers, when imported, are typically from multiple sources and are managed separately from the breeding herd. Thus, the risk posed by importing stockers is similar to the risk posed by fenceline contact with any infected herd. If PIs are imported with the stockers, TIs in the cow-calf herd are modeled to occur through fenceline contact. The probability that infected stockers sharing a fenceline with the breeding herd will cause a TI is modeled as a binomial variable.

**Table 3.1: Distributions used in stochastic model**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Source</b>
<b>baby calf and fetal prevalence</b>	Normal(0.59%,0.08%) Truncate(0,1)	(Caldow et al., 1993; Wittum et al., 2001; Cleveland, 2003)
<b>youngstock prevalence</b>	Normal(0.47%, 0.11%) Truncate (0, 1)	(Howard et al., 1990; Taylor et al., 1995; Fulton et al., 2000; Cleveland, 2003; Givens et al., 2003b; Loneragen et al., 2005; Gnad et al., 2005)
<b>cow prevalence</b>	Normal (0.07%, 0.04%) Truncate (0, 1)	(Smith et al., 2007)
<b>herd prevalence</b>	Normal (10.16%, 2.7%) Truncate (0, 1)	(Wittum et al., 1997; Wittum et al., 2001)
<b>probability of infection from fenceline contact</b>	Pert (6%,47%,83%)	Expert survey*
<b>test sensitivity</b>	Normal (97%, 1.6%) Truncate (0, 1)	(Frey et al., 1991; Mignon et al., 1992; Haines et al., 1992; Ellis et al., 1995; Sandvik and Krogsrud, 1995; Brinkhof et al., 1996; Deregt and Prins, 1998; Graham et al., 1998a; Schreiber et al., 1999; Saliki et al., 2000; Plavsic and Prodafikas, 2001; Grooms and Keilen, 2002; Deregt et al., 2002; Ozkul et al., 2002; Kim and Dubovi, 2003; Cornish et al., 2005; Walz et al., 2005; Kuhne et al., 2005; Kennedy et al., 2006)

\* A panel of 5 veterinarians involved in BVDV research.

The only biosecurity measure included in the model is testing imports for PIs. Options for testing include the testing of adult imports (bulls and heifers), the testing of calf imports, which may include the calves of pregnant imports, and the testing of stockers. The number of PIs detected is based on the number tested and the sensitivity of the test. Test sensitivity is modeled as one estimate for all diagnostic procedures currently in regular use, derived from a meta-analysis of test sensitivity (data not shown) which failed to detect a difference in sensitivity between tests. There was a lack of power in the meta-analysis due to the few published reports of sensitivity calculations; however, with the data available, the sensitivity distributions are not significantly different for the different tests. The model assumes that all imports tested are held in quarantine until test results are obtained, at which point PI animals are removed from the herd. If calves of pregnant imports are tested, it is assumed that the dams of all positive calves will also be tested and that pregnant PIs will be preferentially represented in that testing.

The model was programmed with @Risk 4.5.7 (Palisade Corp, Ithaca, NY), an add-in for Microsoft<sup>®</sup> Excel 2003 (©1985-2003 Microsoft Corp, Redmond, WA)

## ***2. Sensitivity Analysis***

### ***2.1 Materials and Methods***

The sensitivity of the model output to the various input distributions was examined based on a 400 head herd (herds A1-A4 from Table 3.2), with biosecurity strategies M (no testing) and T (testing all imports) from Table 3.3. All imported heifers were modeled to be pregnant. Distributions listed in Table 3.1 were fixed one by one at the mean plus 2 standard deviations, and the mean minus 2 standard deviations to allow examination of the changes observed in output at the levels that could be expected to occur 95% of the time. Since the herd examined does not import cows that prevalence distribution was not included in the analysis.

One output was monitored to indicate the introduction of BVDV to the herd after one year, the likelihood of BVDV introduction (LBI). The LBI is a binomial variable indicating that PIs were imported and/or TIs were caused by contact with stockers. The mean of LBI, therefore, indicates the probability of BVDV introduction in any form.

**Table 3.2: Import profile of herds tested**

<b>Herd</b>	<b>number of breeding females</b>	<b>number of heifers imported</b>	<b>number of bulls imported</b>	<b>number of calves imported</b>	<b>number of stockers imported</b>
<b>A1</b>	400	60	4	4	100
<b>A2</b>	400	60	4	4	0
<b>A3</b>	400	60	4	0	100
<b>A4</b>	400	60	4	0	0
<b>B1</b>	100	15	1	1	100
<b>B2</b>	100	15	1	1	0
<b>B3</b>	100	15	1	0	100
<b>B4</b>	100	15	1	0	0
<b>C1</b>	50	8	1 every other year	1	100
<b>C2</b>	50	8	1 every other year	1	0
<b>C3</b>	50	8	1 every other year	0	100
<b>C4</b>	50	8	1 every other year	0	0

**Table 3.3: Testing strategies**

<b>Strategy</b>	<b>Animals tested</b>
M	None
O	Adult imports (heifers, cows, and bulls)
P	Calf imports and calves of pregnant imports
R	Stockers imported
S	Adult imports, calf imports, and calves of pregnant imports
T	All imports (calves, heifers, cows, bulls, calves of pregnant imports, and stockers)

The simulation was run for 3000 iterations at each level with a fixed random number seed. Distributional effect of each input parameter was calculated by the difference between the value of each output at the extremes of each parameter distribution.

## **2.2 Results**

Results of the sensitivity analysis are shown in Table 3.4. When no imports are tested (strategy M) and stockers are imported (profiles 1 and 3), calf/fetal prevalence and youngstock (heifer, stocker, and bull) prevalence are equally influential distributions on the likelihood of introducing BVDV, but calf/fetal prevalence is more influential when stockers are not imported (profiles 2 and 4) and youngstock prevalence is more influential when all imports are tested (strategy T). When all imports are tested (strategy T), test sensitivity has the largest effect on LBI. Changing the probability of an infection from fence-line contact with stockers has the largest impact of any distribution on LBI when no animals are tested (strategy M). If all animals are tested (strategy T) and stockers are imported (profiles 1 and 3), the likelihood of infection from fence-line contact is a more influential distribution than calf/fetal prevalence, but is slightly less influential than youngstock prevalence.

**Table 3.4: Comparison in the likelihood BVDV is introduced to a 400-head herd in one year when each input distribution is fixed at the mean minus 2 standard deviations and at the mean plus 2 standard deviations.**

Input Distribution	Change in Input Distribution	Difference in the likelihood BVDV is introduced with change in the input distributions							
		All imports are tested (T)				No biosecurity strategy (M)			
		Profile 1*	Profile 2*	Profile 3*	Profile 4*	Profile 1*	Profile 2*	Profile 3*	Profile 4*
calf/fetal prevalence	0.43%- 0.77%	0.011	0.011	0.010	0.010	0.106	0.133	0.101	0.128
youngstock prevalence	0.25%- 0.69%	0.020	0.011	0.021	0.011	0.103	0.025	0.105	0.025
test sensitivity	93.8%- 100%	-0.043	-0.034	-0.040	-0.032				
herd prevalence	4.66%- 15.56%	0.000	0.000	0.000	0.000	0.048	0.058	0.049	0.060
Fenceline infection probability	6%-83%	0.018		0.018		0.179		0.183	

\*Profiles A1-A4 from Table 2a: annual imports into the herd

Profile 1 – 60 pregnant heifers, 4 bulls, 4 calves, 100 stockers

Profile 2 – 60 pregnant heifers, 4 bulls, 4 calves

Profile 3 – 60 pregnant heifers, 4 bulls, 100 stockers

Profile 4 – 60 pregnant heifers, 4 bulls

### ***3. Application of the Model***

#### ***3.1 Materials and Methods***

The model was applied to three herd sizes, as shown in Table 3.2, to represent small medium and large herds. Each herd was modeled to include the importation of baby calves, stockers, both, or neither. Separate simulations were run for each import profile (Table 3.2) to include pregnant heifers only or non-pregnant heifers only. In addition, each simulation was repeated with the biosecurity strategies listed in Table 3.3. Import profiles were designed to

represent average cow-calf herds, in which 15% of animals are replaced annually and all replacements are purchased as heifers.

Two outputs were monitored for one year for each simulation. First, as in the sensitivity analysis, was the likelihood of BVDV introduction, LBI. Second, the number of PIs imported (iPI) was monitored as an indication of the level of risk posed by a specific import profile. The iPI output includes animals imported to the breeding herd, so imported stockers are not included.

Each simulation included 3000 iterations with a fixed random number seed. Convergence to less than 5% variation in the mean and standard deviation was observed for all outputs with less than 3000 iterations. Means were calculated for each of the outputs monitored by @Risk. The effect of any biosecurity strategy was calculated as the difference between the output mean for the base herd (no biosecurity strategy) and the strategy in question. Statistical comparisons were performed using the Student's t-test.

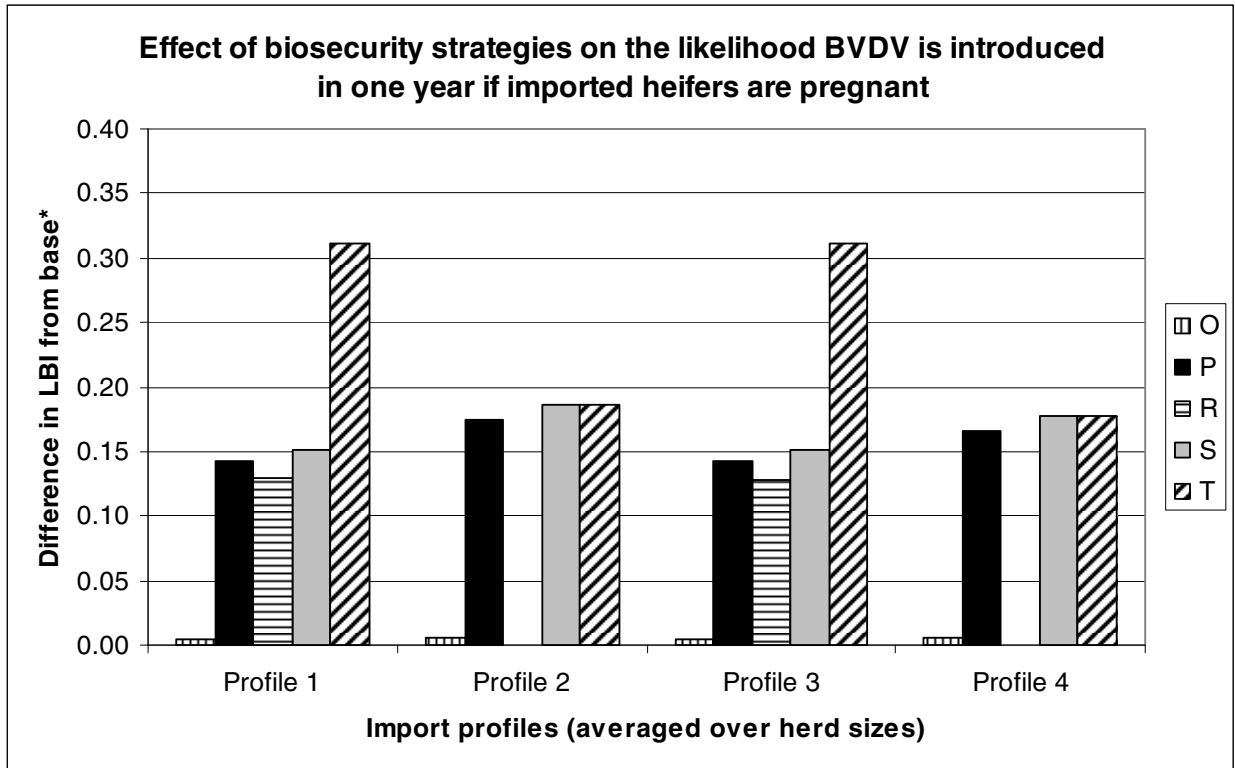
### **3.2 Results**

The effect of changing biosecurity strategies on LBI is shown in Figure 3.1. The proportional change in LBI with each strategy was similar across the three herd sizes for each import profile (with less than 1% difference between herd sizes for any strategy, data not shown), so the effect was averaged across herd sizes. Briefly, testing calves was more efficacious than testing adults when pregnant heifers were imported (Figure 3.1a), but not when non-pregnant heifers were imported (Figure 3.1b). Testing all imports, including stockers, was always the most efficacious (had the greatest impact on decreasing the likelihood of introduction) biosecurity strategy when stockers were imported. When imported heifers were not pregnant, testing stockers alone was more efficacious than testing any other imports (Figure 3.1b, strategy R), but testing the imported calves was more efficacious if the imported heifers were pregnant (Figure 3.1a, strategy P). Testing adults alone was only effective when no calves, pregnant heifers, or stockers were imported (Figure 3.1b, strategy O). Importing stockers or pregnant heifers raised the LBI in all herd sizes and import profiles.



**Figure 3.3.1: Decrease in the likelihood BVDV is introduced to the herd in one year due to biosecurity strategies, when compared to the base value\* for the herd (the mean value if no testing is done), averaged over herd sizes (400-, 100-, and 50-head).**

3.1a) Imported heifers are pregnant

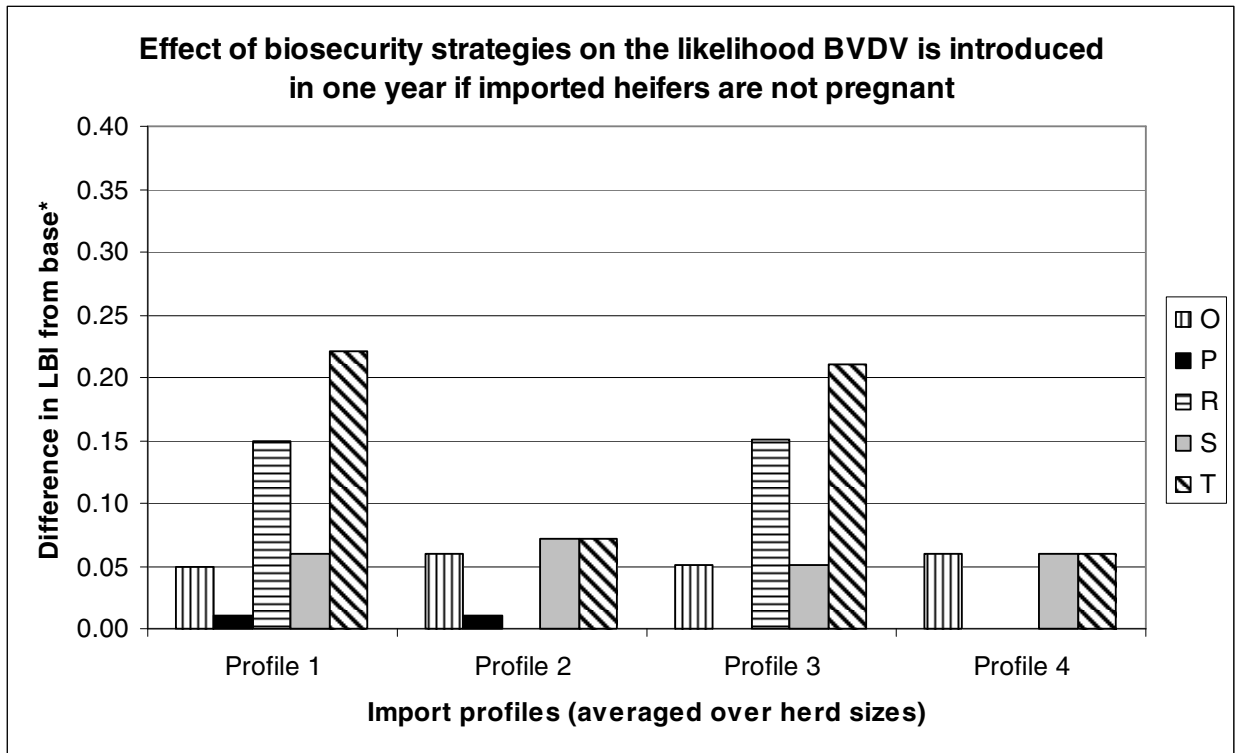


\*base values for likelihood BVDV introduced (LBI) if no testing is used (strategy M):

Herd Size	Profile 1	Profile 2	Profile 3	Profile 4
imports	Heifers, bulls, calves, stockers	Heifers, bulls, calves	Heifers, bulls, stockers	Heifers, bulls
400-head	0.50	0.39	0.49	0.37
100-head	0.29	0.14	0.28	0.13
50-head	0.24	0.08	0.23	0.08

Biosecurity strategies: O (test imported adults), P (test imported calves and calves of pregnant imports), R (test imported stockers), S (test imported adults, calves, and calves of pregnant imports), T (test all imports, including calves of pregnant imports)

3.1 b) Imported heifers are not pregnant



\*base values for likelihood BVDV introduced (LBI) if no testing is used (strategy M):

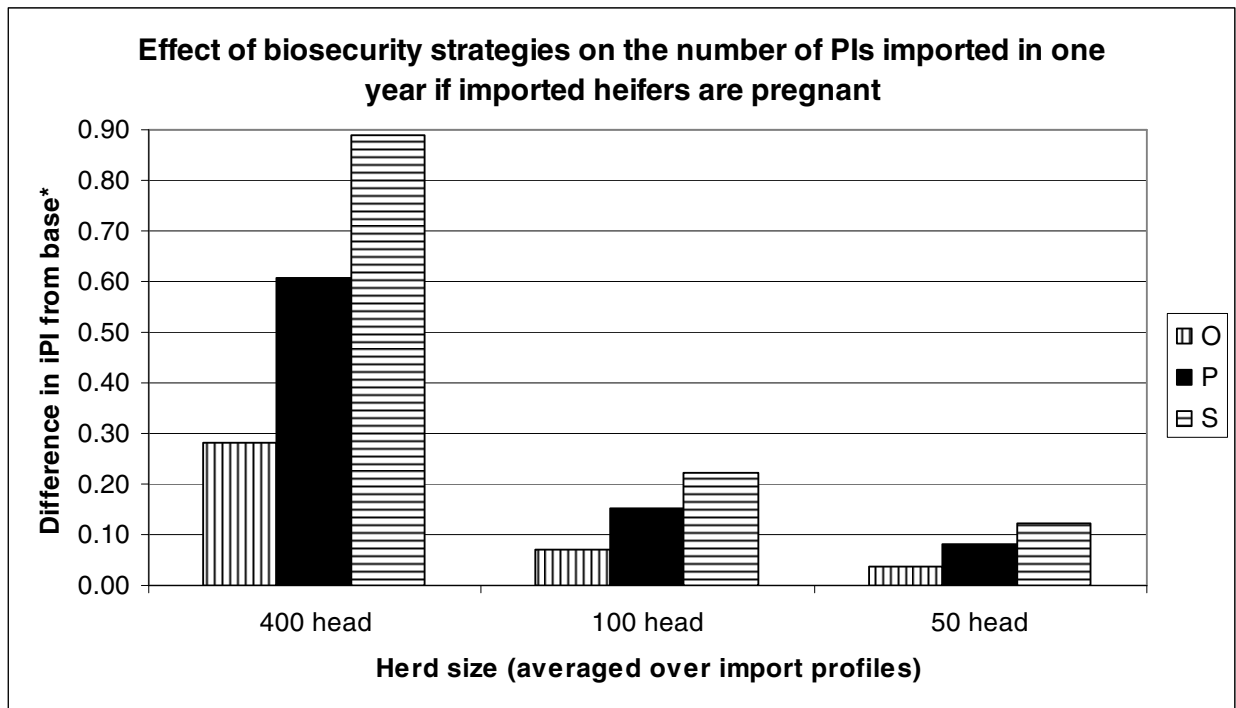
Herd Size	Profile 1	Profile 2	Profile 3	Profile 4
imports	Heifers, bulls, calves, stockers	Heifers, bulls, calves	Heifers, bulls, stockers	Heifers, bulls
400-head	0.28	0.13	0.26	0.11
100-head	0.22	0.07	0.22	0.06
50-head	0.22	0.04	0.21	0.04

Biosecurity strategies: O (test imported adults), P (test imported calves and calves of pregnant imports), R (test imported stockers), S (test imported adults and calves), T (test all imports)

The effect of changing biosecurity strategies on iPI is shown in Figure 3.2. Since stockers could not be responsible for PI introduction in the model the proportional change in iPI with each strategy was similar across the four import profiles for each herd size (with less than 1% difference between import profiles for any strategy, data not shown), so the effect was averaged across import profiles. Comparing the base values for Figure 3.2a to Figure 3.2b, it is seen that importing pregnant replacement heifers increases the mean iPI for all herd sizes and

import profiles. If imported heifers are pregnant, as in Figure 3.2a, testing imported calves, including the calves of the pregnant heifers, is the most effective way to decrease iPI. If no pregnant heifers are imported, as in Figure 3.2b, testing calves has very little effect compared to testing adults.

**Figure 3.2: Decrease in the number of PIs imported in one year, due to biosecurity strategies, when compared to the base value\* for the herd (the mean value if no testing is done), averaged over import profiles (Table 3.2a).**

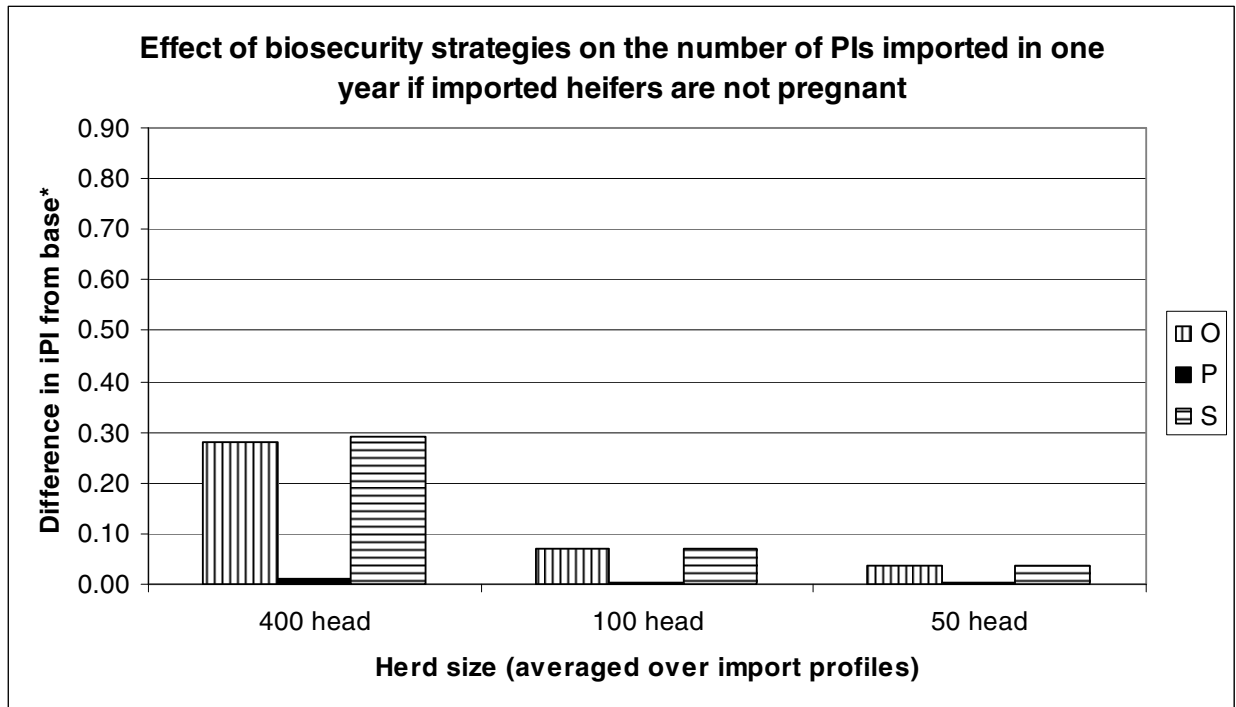


\*base values for the number of PIs imported (iPI) if no testing is used (strategy M):

Herd Size	Profile 1	Profile 2	Profile 3	Profile 4
imports	Heifers, bulls, calves, stockers	Heifers, bulls, calves	Heifers, bulls, stockers	Heifers, bulls
400-head	0.96	0.95	0.94	0.93
100-head	0.23	0.23	0.24	0.22
50-head	0.13	0.13	0.13	0.12

Biosecurity strategies: O (test imported adults), P (test imported calves and calves of pregnant imports), S (test imported adults, calves, and calves of pregnant imports)

2 b) Imported heifers are not pregnant



\*base values for the number of PIs imported (iPI) if no testing is used (strategy M):

Herd Size	Profile 1	Profile 2	Profile 3	Profile 4
Imports	Heifers, bulls, calves, stockers	Heifers, bulls, calves	Heifers, bulls, stockers	Heifers, bulls
400-head	0.34	0.34	0.31	0.31
100-head	0.09	0.09	0.08	0.08
50-head	0.05	0.05	0.05	0.05

Biosecurity strategies: O (test imported adults), P (test imported calves), S (test imported adults and calves)

None of the means for either the LBI or iPI output showed significant differences between biosecurity strategies or import profiles (data not shown).

## Discussion

The model presented here shows that the import profiles of a cow-calf herd impacts both the risk of introducing BVDV to the herd and the efficacy of any testing strategy.

Sensitivity analysis to identify the most influential distributions showed that the impact of a distribution depends on the modeled import profile and biosecurity strategy. The relative quantity of animals imported from a given age category affected the influence of the prevalence distribution for that category if imports were not tested (strategy M). For herds not importing stockers (profiles 2 and 4), calves, including the calves of pregnant heifers, were more influential, likely due to the higher mean prevalence. When imports were tested, test sensitivity became the most influential distribution and the probability of infection from fence line contact became relatively less important for the herds importing stockers. While the distribution for test sensitivity is well-defined from the literature, the fence line infection probability distribution is broad and based on expert opinion. Therefore, it is reassuring to note that the distribution representing more uncertainty is not overly influential in the model if distributions in which we have more confidence are brought to bear.

Importing stockers and allowing fence line contact with the breeding herd increases the risk of introducing BVDV to a cow-calf herd. Importing stockers may overwhelm the effects of other, less risky, behaviors, such as importing a small number of baby calves. If both stockers and pregnant animals are imported, the likelihood of BVDV introduction can be decreased almost equally by either testing strategies involving stockers alone or by testing the calves of pregnant imports; the risk from the heifers and their calves is as high as the risk from the stockers. If imported heifers are not pregnant, however, the risk from stockers is higher than the risk from other imports and testing stockers is much more efficacious than testing other imports; relatively more stockers are imported, so there is an arithmetically greater risk from those animals. In all cases involving imported stockers, however, the best option for decreasing the risk from stockers is testing the imported stockers. This model assumes that imported stockers will be housed adjacent to the cow-calf herd. Managing stockers in non-adjacent pastures or pastures without fence line contact would likely also decrease the risks posed by imported stockers.

The reproductive status of imported heifers, in the herds examined, greatly impacts both the risk of introducing BVDV to the cow-calf herd and the number of PIs imported. Pregnant heifers provide two opportunities for a PI import: the heifer and the fetus. This effectively doubles the number of imports, with the fetal imports coming from a category with a higher prevalence. Testing the calves of pregnant heifers has a greater impact on both LBI and iPI than

testing the heifers, indicating that the majority of the increased risk comes from the fetal imports. Testing the dams of the PI calves, which the model assumes will occur, adds an additional benefit.

In 50- and 100-head herds, the impact of fetal imports on the mean LBI is smaller than the risk posed by importing stockers. This is most likely due to the proportionally higher number of stockers imported compared to heifers in these herd sizes. Import rates in cow-calf operations are variable but herds would not ordinarily import more than 15-20% of the herd inventory as replacement heifers in a given year unless the herd is undergoing significant expansion. Cow-calf herds may import a substantially larger number of stocker cattle as a routine management practices or in years where additional grazing resources are available. The larger number of stockers imported in this model example likely account for the increased risk.

Some cow-calf herds will import a small number of baby calves to raise with their own calves, typically to graft onto cows or heifers that have lost a calf. The number of these calves is generally small, and despite their expected higher prevalence of PI-BVDV infection the small number imported in this model appears to be less of a risk than importing stockers or pregnant animals. The decision to import calves only increased the number of PI's imported slightly, but few calves were imported in the model. The incremental risk associated with importing calves is the same as that of the feti of pregnant animals, which is higher than any others. However, due to the small numbers the risk posed by the calves was masked by the risk associated with other classes of cattle including pregnant heifers and imported stockers. Imported baby calves remain a risk for disease introduction for numerous pathogens and the relative lack of importance here should not be interpreted as a lack of any risk.

The choice of testing strategy depends on the import profile of the herd. Herds importing stockers need to test the stockers to decrease their likelihood of introducing BVDV or alternately prevent fence line contact with the breeding herd. Herds importing pregnant heifers must test the calves of those heifers to decrease their risk. Testing calves of imported pregnant heifers, alone or in combination with testing adults, decreased the risk of introducing BVDV and the number of imported PIs for any herd in which pregnant heifers were imported. Testing only adult imports was the most effective strategy for decreasing risk only when no pregnant cattle, calves or stockers were imported. These results support that if pregnant females are imported, testing the calves of those imports is necessary for controlling the risk of BVDV. Testing strategies assume

perfect quarantine of all imports until test results are known; if this is not possible, the model will be overly optimistic as to the efficacy of testing.

The model simulation results presented here do not examine the risks posed by fenceline contact or communal pasture with other cow-calf herds, although the risk from fenceline contact is approximated by the risk from importing stockers. These are both possible risks to a herd, and should be considered when choosing a full biosecurity strategy.

Due to the difference in management practices between cow-calf herds and feedlots, it is not possible to directly compare the results of this model with the only previously published model for BVDV introduction to a beef herd (Larson et al., 2002). However, the findings of that study indicated that testing individual animals became less cost-effective as prevalence decreased, which is similar to the findings of the sensitivity analysis here, which showed that increasing prevalence increased risk to a greater extent when animals were not tested. Results here indicate that the number of imported PIs decreased more noticeably with a decrease in youngstock prevalence than with an increase in test sensitivity. The results of Larson et al. may also be supportive of the findings presented here showing that testing is more effective if it is carried out in age groups with higher prevalence distributions, such as calves.

This model assumes that test specificity is 100%. While imperfect specificity does not affect the number of PI animals detected by the test, it does result in a number of non-PI animals to be culled with the PIs. This will impact a herd through the lost production of the culled import, as well as through any genetic improvement and production value that animal could have provided to the herd.

The findings of this model will be useful to cow-calf herds interested in controlling risk of BVDV importation. It is not feasible for many herds to maintain genetic improvements or expansion plans with a completely closed herd. Understanding the risks posed by import choices and the best strategies for counteracting that risk is essential to effective disease control. Importing stockers has often been advised as grazing management and economic risk-mitigation strategy, but this model shows that the BVD disease risks associated with stockers can be high. These risks may be controlled with testing or by preventing fence line contact with the breeding herd. The model also suggests that importing pregnant heifers is a greater risk to the herd than non-pregnant heifers, but that this risk can be mitigated by testing the calves of the imported heifers and culling positives before breeding season. The model has been designed to provide

output relevant to specific farm situations and should prove to be a useful tool in the decision-making process for cow-calf operations.



## **CHAPTER 4 - A risk-analysis model for the spread of BVDV in naïve cow-calf herds after introduction**

### **Abstract**

A stochastic SIR model was developed to simulate the spread of bovine viral diarrhea virus (BVDV) through a cow-calf herd and observe the effect of the virus on abortions, early embryonic deaths, fetal malformations, and calf morbidity and mortality. The model was examined with three herd sizes (400-, 100-, and 50-head) and four control strategies (no intervention, vaccination of breeding stock, testing and culling all calves pre-breeding, and both vaccination of adults and testing of calves). Vaccination and testing combined most decreased the effects of BVDV and the persistence of the virus in the herd. Testing and culling calves pre-breeding alone significantly decreased the duration of the outbreak, but was not always more effective than vaccination alone at decreasing the impact of the virus on the herd.

### **Introduction**

Bovine viral diarrhea virus (BVDV) is a common disease of US cattle herds (Houe et al., 1995b; Paisley et al., 1996; Chase et al., 2003). Reproductive disorders are often seen, such as abortion (Fredriksen et al., 1998), decreased conception rate (Houe and Meyling, 1991a; McGowan et al., 1993a; McGowan et al., 1993b; Larsson et al., 1994; Wittum et al., 2001), early embryonic death (EED) (McGowan et al., 1993a; McGowan et al., 1993b), and fetal defects (Munoz-Zanzi et al., 2003; Ellsworth et al., 2006). In addition, BVDV can cause immunosuppression, which leads to increased disease incidence (Castrucci et al., 1992; Bjorkman et al., 2000; Kozasaa et al., 2005). The cost to producers and the livestock industry, from increased treatment expenses, calf and pregnancy loss, and decreased weight gain (Wittum et al., 1994; Gunn et al., 1998; Bennett et al., 1999; Larson et al., 2002), justifies research on control measures.

The primary source of BVDV spread in a herd is commonly believed to be persistently infected animals (PIs) (Houe, 1999; Niskanen et al., 2002b). Fetal infection

between 40 and 125 days gestation leads to a persistent infection (Stokstad and Loken, 2002) and they shed virus for life through oculonasal discharge and other secretions (Confer et al., 2005).

Many models have dealt with the spread of BVDV in dairy herds (Innocent et al., 1997a; Innocent et al., 1997b; Cherry et al., 1998; Viet et al., 2004a; Viet et al., 2004b; Viet et al., 2005; Viet et al., 2006; Ezanno et al., 2007), but only one model exists for spread of BVDV through a cow-calf herd (Cleveland, 2003). Due to management differences between dairy and beef operations, the latter is the only model in the published literature that is applicable to cow-calf operations. In particular, the limited breeding season of beef herds limits the risk period for BVDV infection leading to the birth of PI animals. This allows for targeted control measures, such as testing and culling calves for viremia before the breeding season. However, it also increases the risk to the herd at this time period, as a greater proportion of dams will be in the risk period at one time than on a dairy, when breeding is usually spread out over the course of a year.

Cleveland designed a stochastic modified Reed-Frost model to determine the efficacy of removing PI animals from a herd of fixed size and structure. While this model was quite useful in identifying possible testing strategies for decreasing BVDV infections, it did not consider either vaccination or the cost-effectiveness of the control strategies. The purpose of this study was to develop a stochastic model to simulate the spread of BVDV through a cow-calf herd and the impact of the virus on factors of economic interest, as well as to examine the efficacy of vaccination and/or testing and culling calves before breeding on decreasing viral impact.

## **Model Building**

### ***1. Developing the model***

A stochastic modified Reed-Frost mass-action model was developed to follow the spread of BVDV through a cow-calf herd. Variations in the parameters involved in transmission of the virus, control measures, and sequelae of infection were modeled with the distributions listed in Table 4.1. Outputs were monitored over a 10-year period, with no intra-year correlations in distributions. The stochastic model was developed with @Risk 4.5 (Palisade Corp, Newfield, NY), an add-in for Excel<sup>®</sup> 2003 (Microsoft Corp, Redmond, WA).

**Table 4.1: Parameter distributions used**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Reference(s)</b>
R <sub>0</sub> when PIs are present	Pert (5,7,12)	Personal opinion
R <sub>0</sub> from fence-line contact	Pert (1.2,3,5)	Personal opinion
Vaccine Efficacy	Pert (0.42,0.845,1)	(Brownlie et al., 1995; Cortese et al., 1998a; Patel et al., 2002; Zimmer et al., 2002; Dean et al., 2003; Kovacs et al., 2003; Fairbanks et al., 2004; Brock et al., 2006; Ficken et al., 2006a; Ficken et al., 2006b)
Abortion Rate	Pert (1.7%,10%,25%)	(Fredriksen et al., 1998; Hassig and Lubsen, 1998)
TI Mortality Rate (the percentage of all TI calves dying of BVDV in one year)	Pert (1%,5%,52%)	Expert survey*
TI Case Fatality Rate (the percentage of calves morbid from TI that will die in one year)	Pert (1%,7%,15%)	Expert survey*

**Table 4.1 (cont.)**

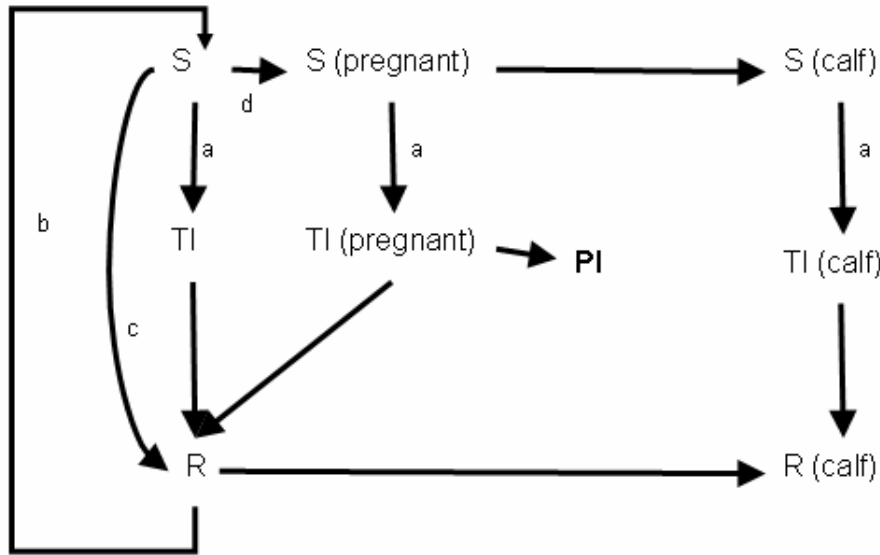
<b>Parameter Description</b>	<b>Distribution</b>	<b>Reference(s)</b>
TI Morbidity Rate	$(TIMortalityRate) / (TICaseFatalityRate)$	Expert survey*
PI mortality (the percentage of all PI calves dying of BVDV before weaning in one year)	Pert (10%,33%,100%)	Expert survey*
PI Case Fatality Rate (the percentage of PI calves morbid from BVDV that will die in one year)	Pert (40%,75%,100%)	Expert survey*
PI Morbidity Rate	$(PIMortalityRate) / (PICaseFatalityRate)$	Expert survey*
weight lost by morbidity	Normal (15.9, 3.5) Truncate (0, )	(Wittum et al., 1994)
Vertical transmission risk	Normal (81%, 0.7%) Truncate (0, 1)	(Stokstad and Loken, 2002)
probability of an EED due to infection during the risk period	Normal (16%,8%) Truncate (0,1)	(McGowan et al., 1993a; McGowan et al., 1993b)
probability congenital defect	Pert (3.2%,13.6%,30%)	Expert survey*
Duration of immunity from transient infection	Binomial(1, 50%) for duration lasting 2 years, all others last 1 year	Personal opinion

\* A written survey of 5 veterinarians with field and research experience with BVDV.

The Reed-Frost model, shown schematically in Figure 4.1, follows three interconnected populations of animals: non-pregnant females, pregnant females, and calves. The number of animals in each category is calculated every 21 days over the course of the calving and breeding

season; the model assumes that transient infections occur only when calves are present in the herd, the period between birth and weaning. The model uses a Markov Chain process to simulate 10 years within the herd. The herd is assumed to be entirely naïve at the beginning of year 1.

**Figure 4.1: Schematic of the Reed-Frost Model**



- S: susceptible animals
- TI: transiently infected animals
- R: resistant animals
- PI: persistently infected animals
- a: transient infection from contact with a PI
- b: loss of immunity
- c: immunization through vaccination
- d: conception

In any group, transfer of individuals from susceptible to transiently infected (a) occurs according to the equation:

$$TI = S * \left( 1 - \left[ 1 - \frac{R_0}{N-1} \right] \right)^{PI}$$

where  $R_0$  is the reproductive rate,  $N$  is the total number of animals in the breeding herd (the sum of the total number of breeding females and the number of calves born at that time period), and  $PI$  is the number of PI animals present in the herd during that time period.

The transfer from the non-pregnant category to the pregnant category is based on a standard conception rate for the length of the herd breeding season (either 60 or 100 days). Infections in pregnant animals are followed in cohorts based on the time period of their conception, allowing calculation of the number of infections during risk periods for EEDs and PIs.

On an annual basis, the number of dams immunized by vaccination is calculated and added to the resistant category, accounting for vaccine efficacy. The number of susceptible dams at the beginning of the year is the difference between the total number of breeding females and the number of resistant dams. The number of susceptible calves added to the herd in each 21 day time period is based on the number of susceptible dams expected to have calved in that period, based on their conception cohort from the previous year. Similarly, the number of PI calves added to the herd in each 21 day calving period is based on the number of dams that were modeled to have produced a PI calf, according to time of conception in the previous breeding season. Persistently infected calves of imported animals were added to the herd randomly throughout the calving period.

The number of resistant cows and calves in any given time period is the sum of the number of immunized animals (if vaccine is given) and the number of animals in that age category previously infected. Immunity from a natural infection lasts at least 12 months in the model, with a binomial process to model the persistence of immunity for a further 12 months in, on average, half the animals. Immunity from vaccination is assumed to last only 12 months, with the annual number of effectively immunized animals based on the number vaccinated and the vaccine efficacy based on available published estimates of efficacy in preventing PI in cows.

## ***2. Sensitivity Analysis***

### ***2.1 Materials and Methods***

The sensitivity of the model output to the various input distributions was examined based on a 400-head herd to which one PI calf was introduced in year 1. The analysis was performed with each of the four control strategies considered: doing nothing, testing of every calf in the

herd and culling PI calves pre-breeding , vaccination of all breeding animals, and both vaccinating breeding animals and testing and culling calves pre-breeding. To allow examination of the changes observed in output at the levels that could be expected to occur 95% of the time, normal distributions listed in Table 4.1 were fixed one by one at the mean plus 2 standard deviations and the mean minus 2 standard deviations. In the case of triangular distributions, the minimum and maximum were used as upper and lower values. The effect of the changing distributions was measured as the change in the mean number of endemic PIs, that is, the number of PI calves born due to infections in the herd after 10 years, a measure of the spread of infection in the herd. The difference between the mean number of endemic PIs at the extremes of the distribution was calculated. Each simulation was run with 3000 iterations and a fixed random number seed.

## **2.2 Results**

The results of the sensitivity analysis are shown in Table 4.2. Distributions listed in Table 4.1 that had no direct link to the number of endemic PIs (TI mortality rate, TI and PI case fatality rate, abortion rate, EED rate, and the amount of weight lost due to morbidity) were not included in the analysis. When no control program was in place, PI mortality rate was the most influential distribution. When vaccination was used for control, alone or in combination with testing, vaccine efficacy was the most influential. When testing and culling calves before breeding was the only control strategy, test sensitivity was the most influential.  $R_0$  was one of the most influential distributions in every scenario.

**Table 4.2: Comparison in the number of PIs born in a 400-head herd in ten years when each input distribution is fixed at the mean minus 2 standard deviations and at the mean plus 2 standard deviations (normal distributions) or at the minimum and maximum values (pert distributions).**

Input Distribution	Change in Input Distribution	Difference in the number of PIs born in a herd in 10 years with change in the input distributions			
		No control	Vaccination	Test-and-cull calves pre-breeding	Vaccination and test-and-cull calves pre-breeding
<b>R<sub>0</sub></b>	5-12	-51.47	11.26	0.22	0.05
<b>Vaccine Efficacy</b>	42%-100%	0	-75.74	0	-0.12
<b>PI mortality rate</b>	10%-100%	-76.79	-24.23	-0.22	-0.03
<b>Vertical transmission rate</b>	79.6%-82.4%	1.60	0.62	0.01	0.0003
<b>Test Sensitivity</b>	93.8%-100%	0	0	-0.65	-0.06

### ***3. Model Application***

#### ***3.1 Materials and Methods***

To assess the performance of the model, simulations were run for three herds, with 50, 100, and 400 head. Virus was introduced to each herd as a single PI calf imported in year one, after which the herd was assumed to be closed in order to determine the impact of a single introduction. Each herd was simulated once for each of the control options built into the model: no control program, vaccination, testing and culling calves pre-breeding, and vaccination and testing and culling calves pre-breeding. A herd with no control program, with no interventions taken, was used to calculate the base value. Vaccination indicates vaccinating all breeding



females. Testing and culling calves pre-breeding denotes testing all calves in the herd and removing any PIs detected before the beginning of the breeding season. The results of these simulations were collected for the numbers of endemic PIs, abortions, calf morbidities, and calf mortalities for 10 years after BVDV introduction. In addition, the annual probability of the presence of at least one PI in the herd was monitored to enable the calculation of clearance rates.

Each simulation was run with 3000 iterations and a fixed random number seed. Means, percentiles, and standard deviations were calculated automatically in @Risk. 95% confidence intervals around the means were calculated with the Student's t distribution. 95% confidence intervals around the percentiles were calculated with the normal Z distribution.

### **3.2 Results**

Convergence was observed in fewer than 3000 iterations for all simulations, measured as less than a 5% change in output mean, standard deviation, and every 5<sup>th</sup> percentile.

None of the means for any output parameters (endemic PIs, abortions, calf morbidity, or calf mortality) are significantly different between any control programs for any herd size (data not shown). The percentiles of certain values, however, are significantly different based on comparison of 95% confidence intervals, as is shown in Figures 4.2 through 4.5. First-order dominance was observed for all output parameters with a combination of testing and culling calves pre-breeding and vaccinating breeding animals. The survival curves for BVDV in the herd are shown in Figure 4.6. No significant advantage is observed for adding vaccination of adult animals to testing and culling of calves pre-breeding.

## **Discussion**

The strength of this model is its flexibility, its ability to be adapted to different herd sizes and management procedures. However, some general lessons can be drawn from the results of the model at this point.

The relative importance of the distributions in the model, as seen in the sensitivity analysis, provides useful insight regarding the impact of different control measures. An increase in  $R_0$  from the minimum to the maximum value decreases the number of endemic PIs when no control program is in place, but the opposite is true if there is some form of control program used. This would imply that, if natural infection is allowed to occur, a highly infectious strain would move through the herd quickly, giving natural immunity to dams before they are at risk

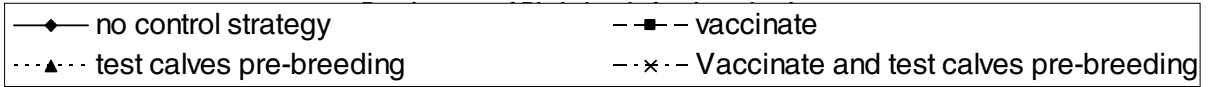
for producing a PI fetus. The high impact of changing vaccine efficacy from its 5<sup>th</sup> to its 95<sup>th</sup> percentile is due in part to the width of its distribution; compared to the vertical transmission rate, the variation is more extreme and would be expected to produce a bigger effect. This wide distribution is meant to capture the natural variability of vaccine efficacy, which depends on the strain of viral challenge, the vaccine used, and several management factors. The PI mortality rate and  $R_0$  distributions are also wide in order to account for similar natural variability, as well as uncertainty, so their influential behavior is expected.

Increasing PI mortality rate decreases the number of PIs born to the herd. As PI mortality is calculated by the model every three weeks, a high mortality rate results in PIs being removed from the herd quickly, before any or all new fetuses have reached the risk period for persistent infection. This model, then, supports anecdotal evidence that BVDV outbreaks are less severe if PI calves die young.

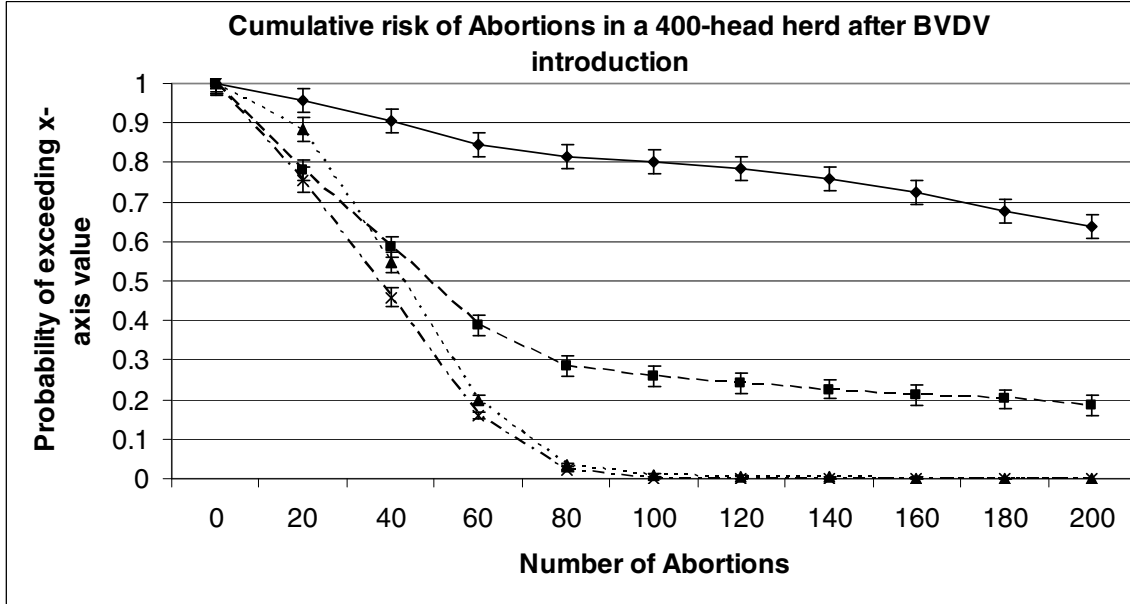
There are no significant differences in the means of any output measures under any of the control strategies. This is understandable, as the variability and uncertainty in the model allow for a wide range of results, which is realistic when compared to field experience of BVDV outbreaks. The tails of the output distributions, however, are significantly different based on comparison of probabilities, implying that control programs will decrease risks, and specifically decrease the risk of large effects. This underscores the importance of using stochastic models to determine disease risk; a deterministic model would lose the distinction between control strategies. This is especially important when uncertainty and/or natural variability is important to an input parameter. For example, both vaccine efficacy and the  $R_0$  of BVDV are reliant on outside factors that vary considerably between herds, such as nutrition, stocking density, strain of the infecting virus (virulence and similarity to the vaccine strain) and other management factors. As seen in the sensitivity analysis, both of these distributions are highly influential on the output of the model. Using stochastic methods to account for the variability involved allows for a more sensitive model, able to predict the range of expected outcomes from BVDV introduction.

**Figure 4.2 Cumulative distributions for the number of abortions observed in 10 years after BVDV introduction in a (a) 400-head herd, (b) 100-head herd, and (c) 50-head herd.**

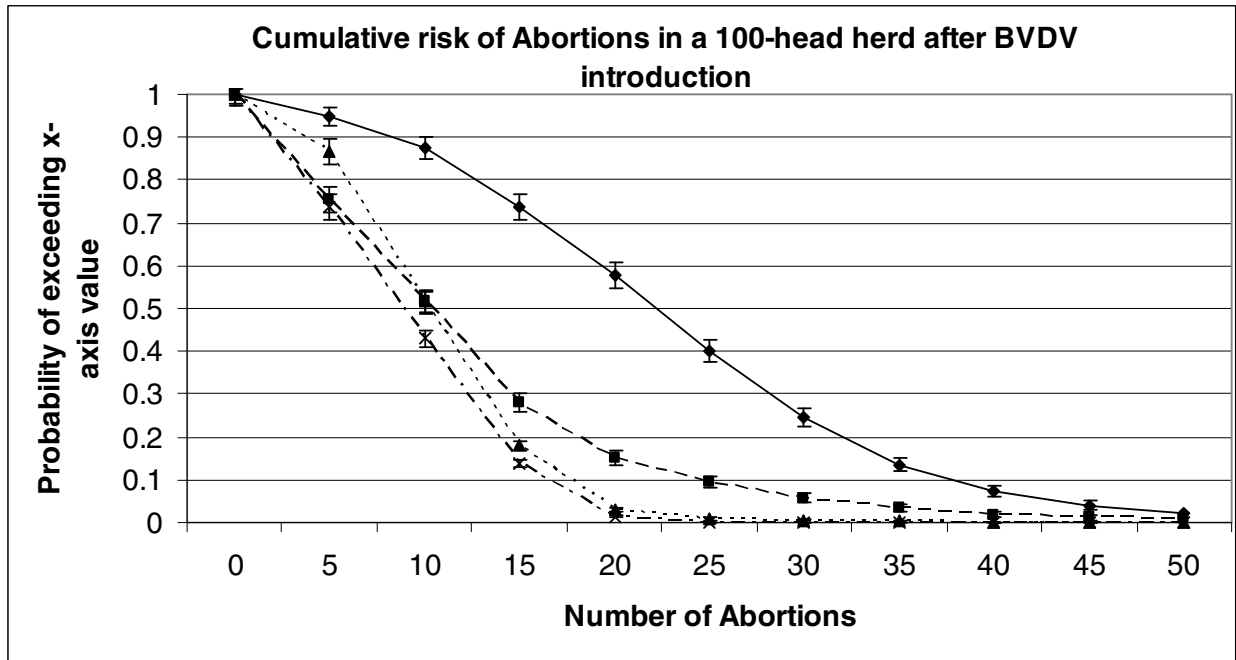
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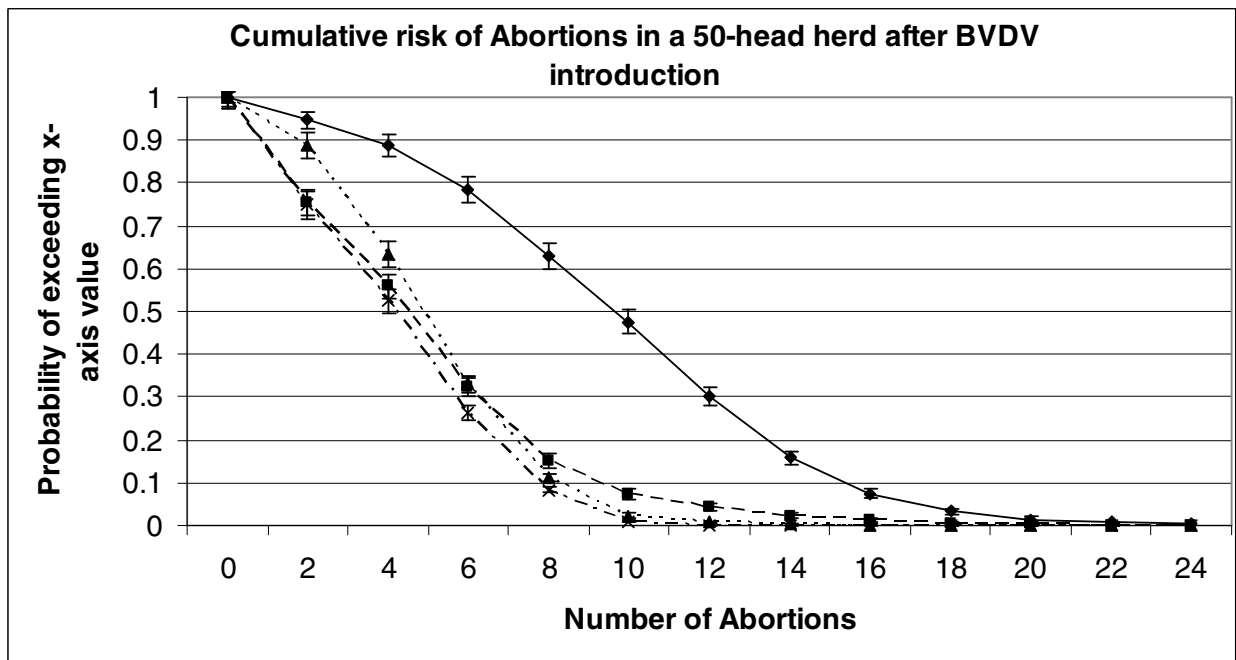
2(a)



2(b)



2(c)

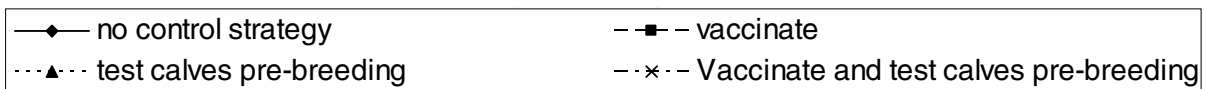


The results presented here indicate that testing all calves in the herd and culling PI calves pre-breeding greatly decreases the risk of the outputs examined. In 400- and 100-head herds, abortion risk is decreased more by test-and-cull strategies than by vaccination alone (Figure 4.2). In 50-head herds, however, there is mixed dominance between vaccination alone and testing and culling calves alone; vaccination significantly decreases risk of low levels of abortions, but

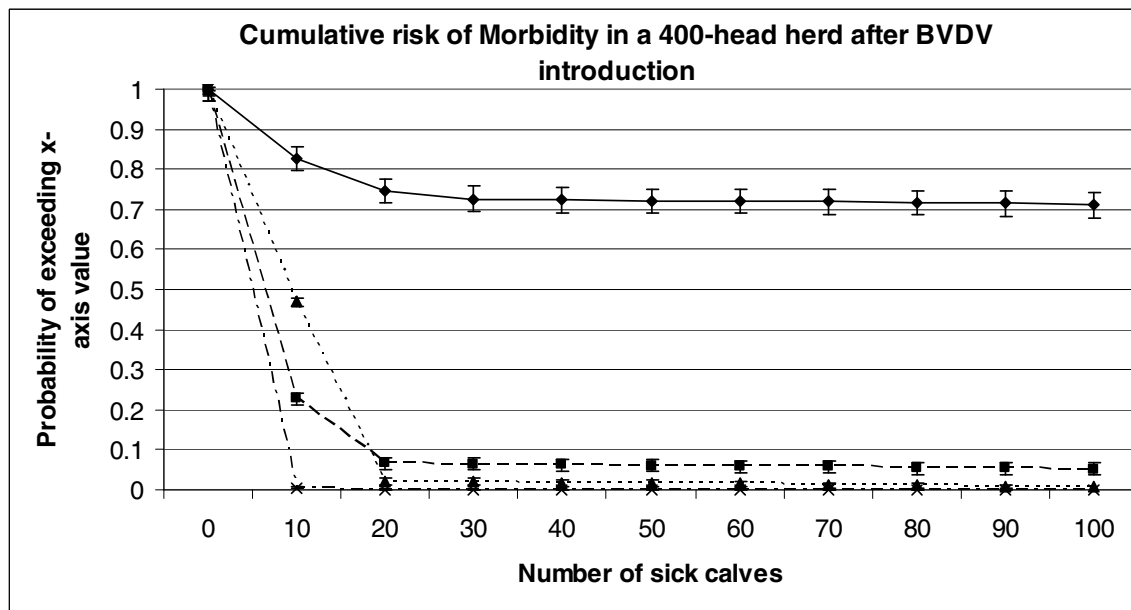
testing and culling calves pre-breeding is more effective at preventing larger abortion storms. Combining both testing and vaccination shows first-order dominance for all herd sizes, as would be expected. The effect of herd size is most likely related to the smaller pool of susceptible animals for infection and the smaller number of PIs in 50-head herds. Vaccination would lower the effective  $R_0$  ( $R_E$ ) below the level necessary to maintain an infection in most cases, but in some situations, in which vaccine efficacy was low or  $R_0$  was high, herd immunity would fail and it would be necessary to remove the source of the infection, the PI calves. In small herds, the probability of all PI calves dying without removal is higher because there are fewer PIs to begin with, usually only one or two; in larger herds, it is more likely that one of the many PIs will survive the high mortality rate to infect more animals and would need to be removed with a test-and-cull program.

**Figure 4.3: Cumulative distributions for the number of morbidities observed in 10 years after BVDV introduction in a (a) 400-head herd, (b) 100-head herd, and (c) 50-head herd.**

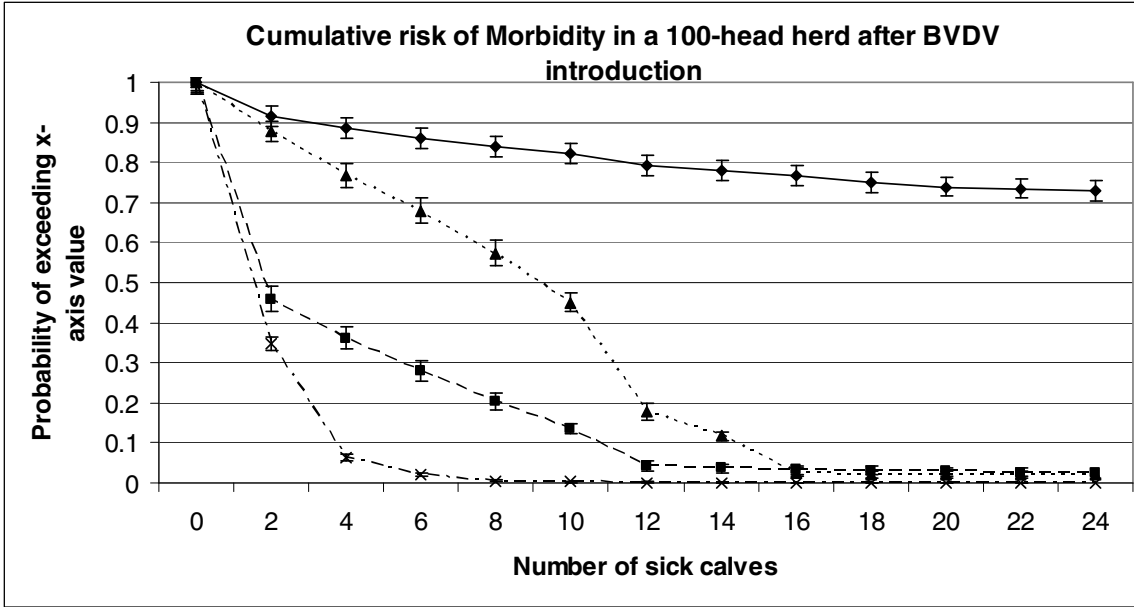
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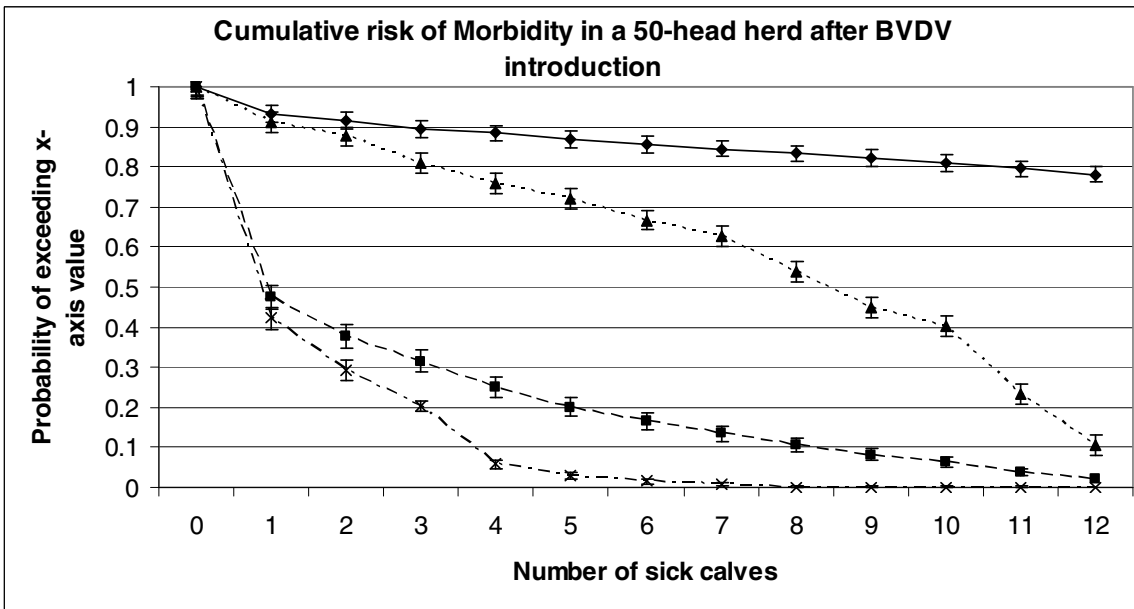
3(a)



3(b)



3(c)

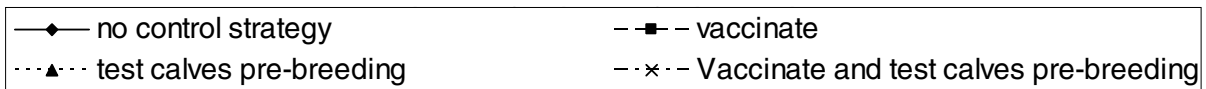


In contrast to abortions, the risk of calf morbidity and mortality (Figures 4.3 and 4.4) is usually decreased more by the vaccination of breeding animals than by the test-and-cull strategy alone, although testing is a significant improvement over doing nothing. In 400-head herds, vaccination alone and test-and-cull alone decrease the risk of mortality equally (Figure 4a). The model counts morbidity and mortality during the calving season, before endemic PIs would be detected by testing and culled, thereby allowing the PIs to produce TI-associated morbidity and

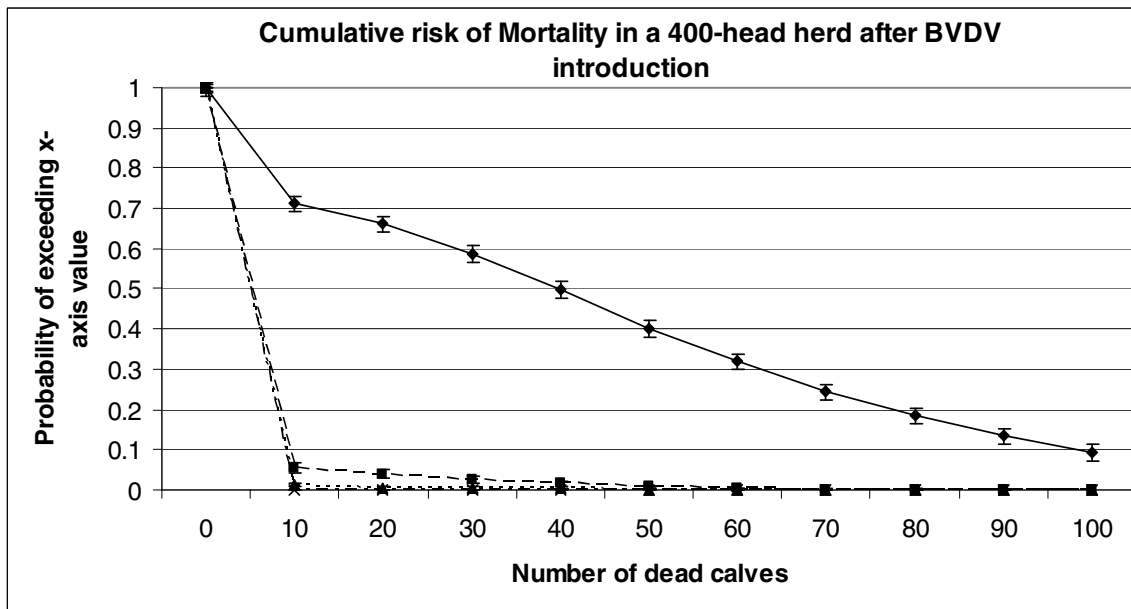
mortality. Vaccination, however, can decrease morbidity and mortality in calves through the passive immunity conferred by vaccinated dams, which protects the calves from infection before the breeding season starts and PI calves would be culled. An alternative to this could be to test and remove PI calves at birth, but this model does not address that option as testing and culling calves is more commonly performed at branding, just prior to the breeding season. In 400-head herds, vaccination may not be as effective as in the smaller herds because of the larger pool of susceptible animals. These animals can maintain an infection by supplying new PIs to infect subsequent calf crops, which is less likely when only one or two animals remains susceptible every year compared to 8 to 10.

**Figure 4.4: Cumulative distributions for the number of mortalities observed in 10 years after BVDV introduction in a (a) 400-head herd, (b) 100-head herd, and (c) 50-head herd.**

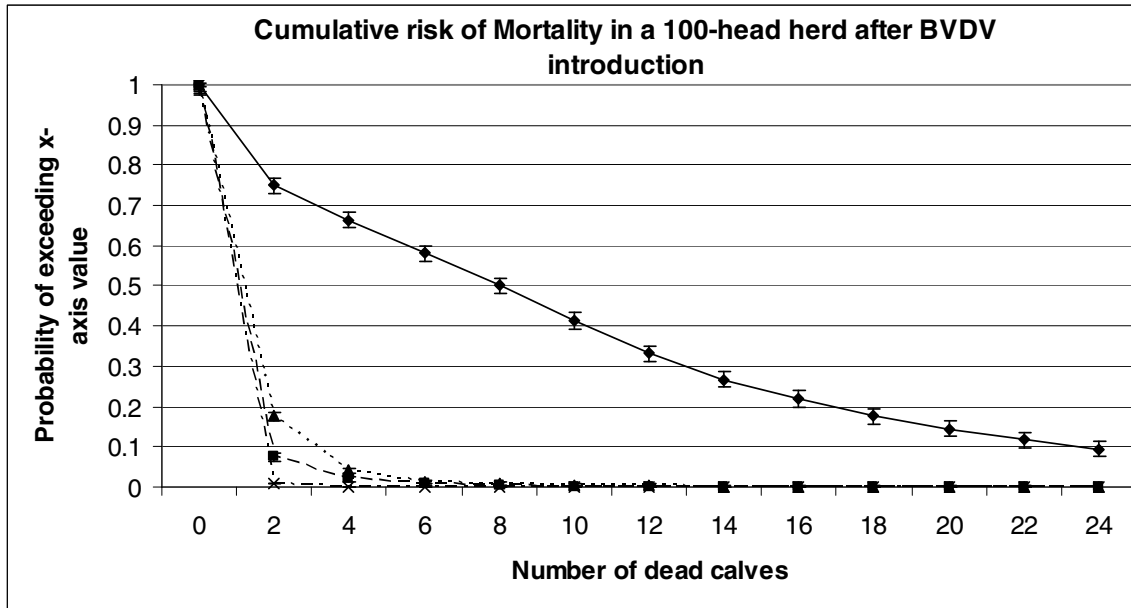
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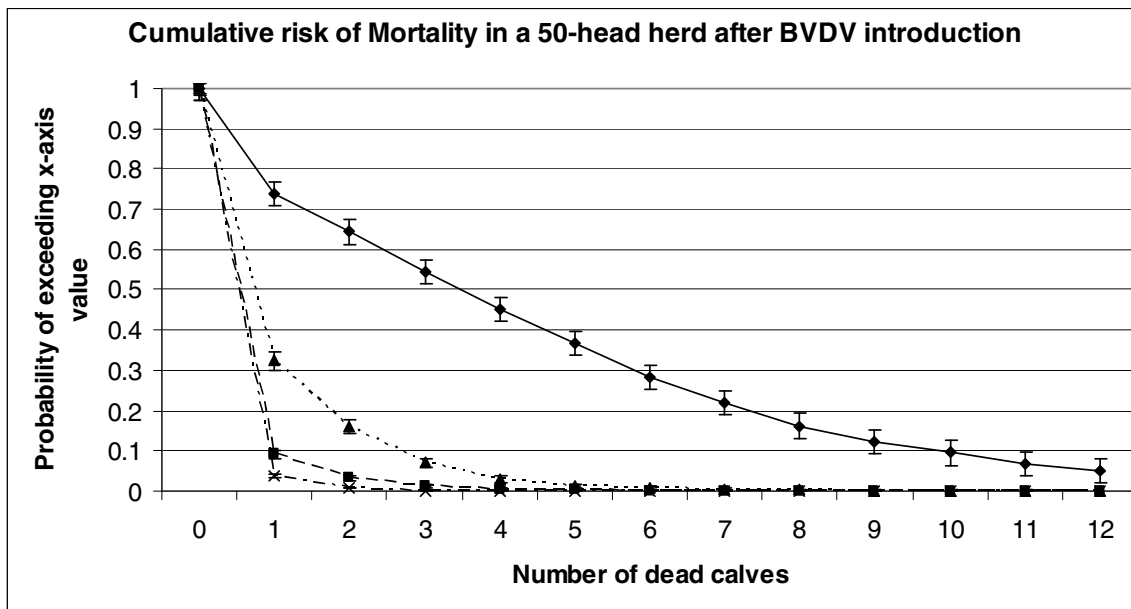
4(a)



4(b)



4(c)



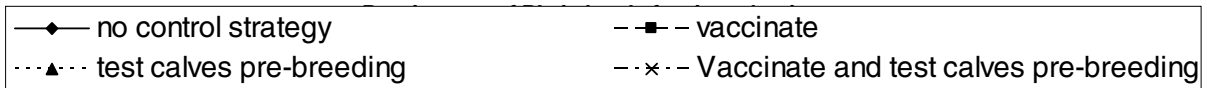
In the case of endemic PIs (Figure 5), there is no significant decrease, based on comparison of 95% confidence intervals, in the cumulative probability distribution of endemic PIs when vaccination is added to a test-and-cull strategy for calves compared to a test and cull strategy alone. Vaccination alone does significantly decrease risks compared to no control, but has significantly higher risks than a calf test and cull strategy alone. This effect appears to be



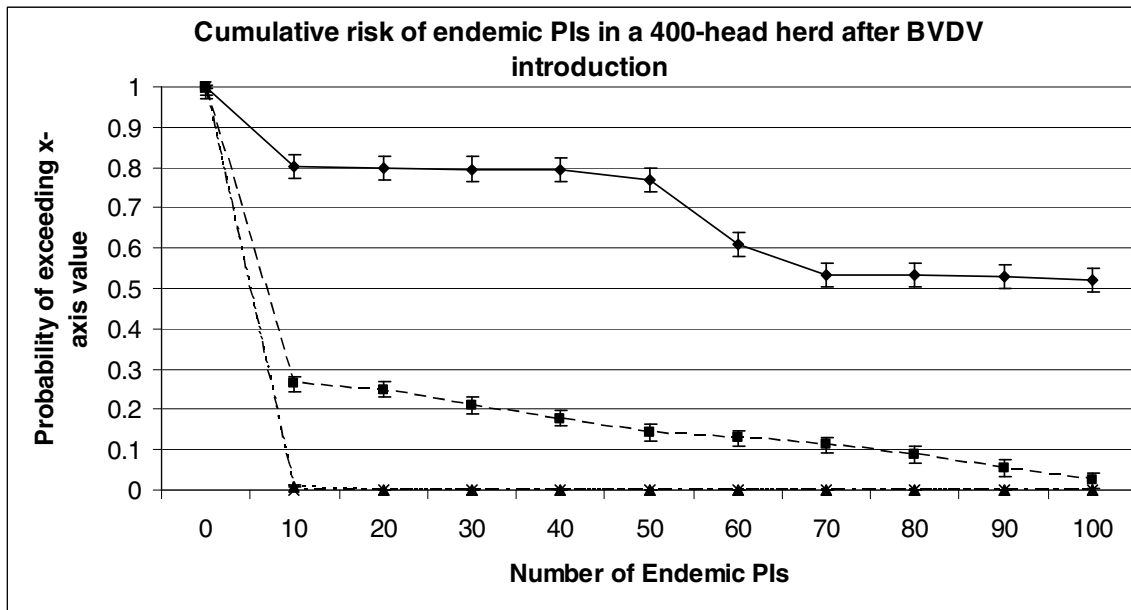
consistent across herd sizes. This strategy, of course, has calves tested and removed from the herd before they can expose gestating cows in the PI risk period (40 to 125 days gestation). A sensitive testing strategy for removal to prevent exposure and infections is more effective than a vaccination program subject to variable efficacy.

**Figure 4.5: Cumulative distributions for the number of endemic PIs observed in 10 years after BVDV introduction in a (a) 400-head herd, (b) 100-head herd, and (c) 50-head herd.**

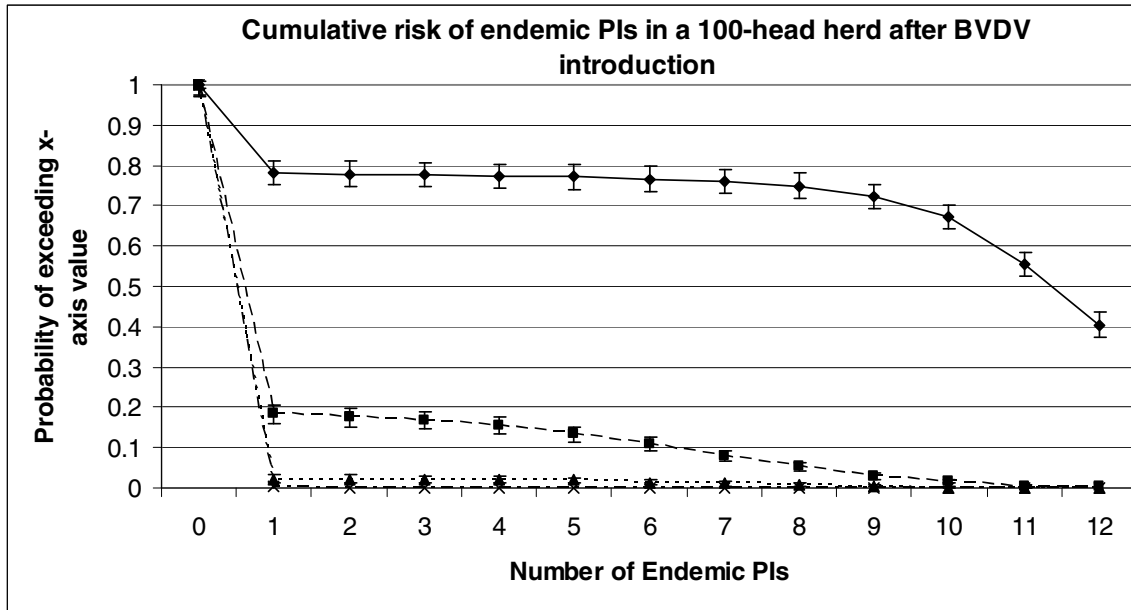
Key:



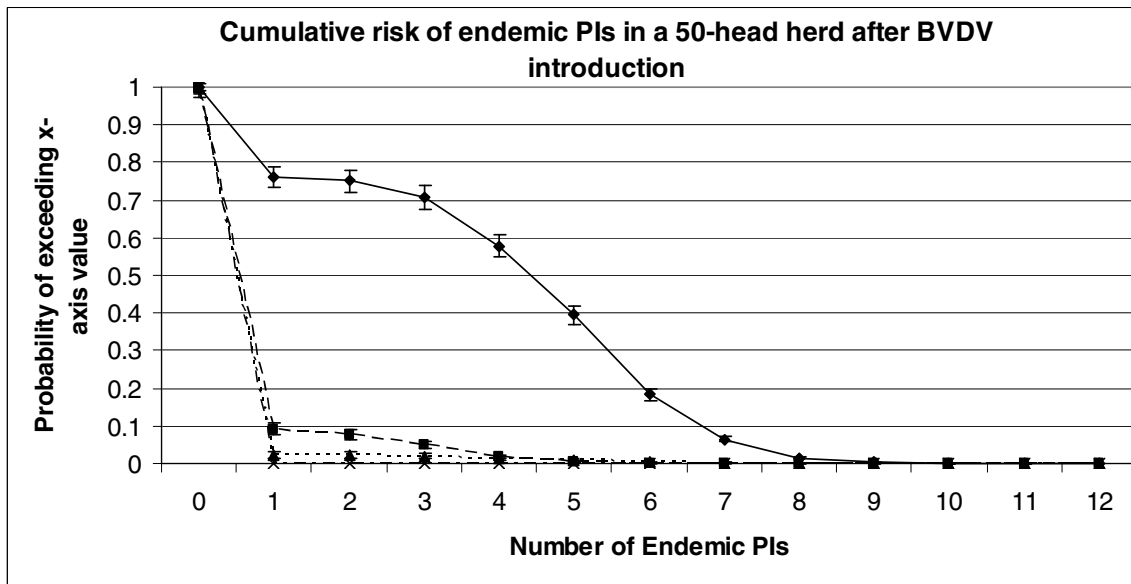
5(a)



5(b)



5(c)

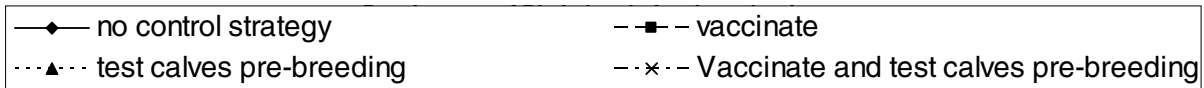


Of interest to producers is how long an outbreak might last, once BVDV is introduced to their herd. The survival curves in Figure 4.6 indicate that testing and culling calves pre-breeding significantly decreases the probability of an outbreak of long duration. By removing PIs from the herd before the conception and infection of new fetuses, the herd is able to clear the virus

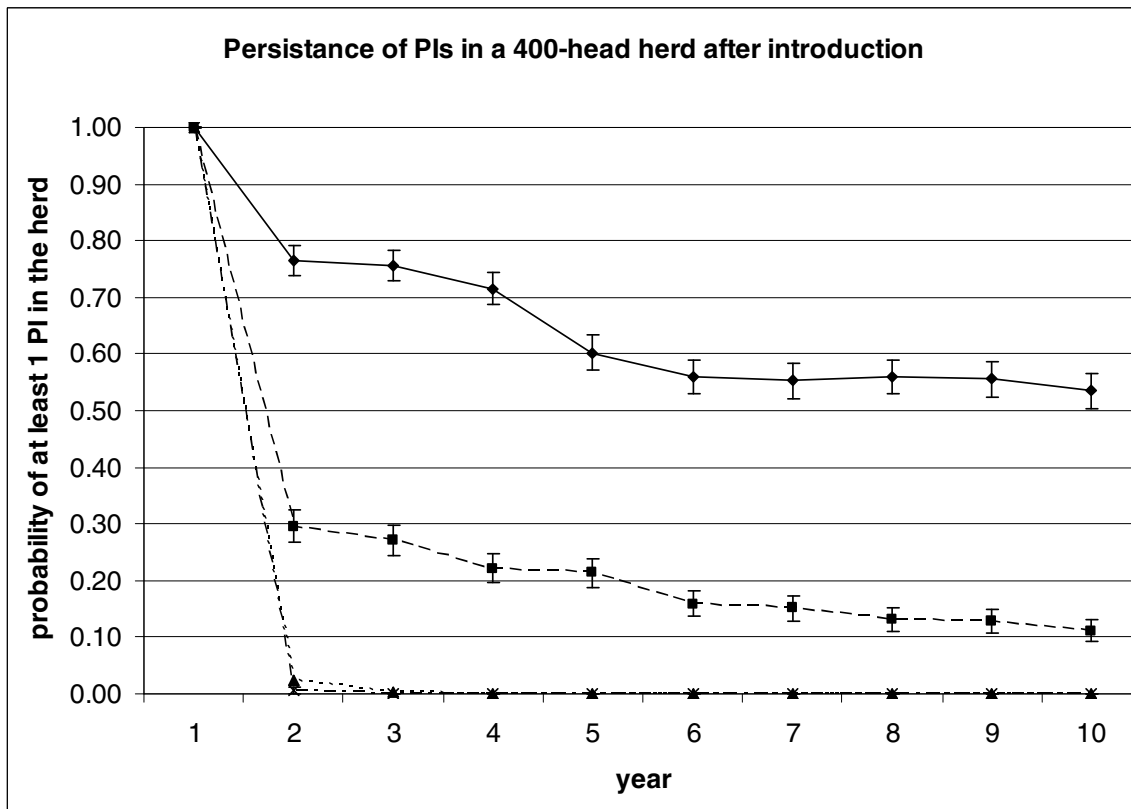
more quickly. As suggested by the results discussed above, test-and-cull methods were able to decrease the number of PIs present in the herd, the source of infection by BVDV.

**Figure 4.6: Persistence of PIs in herd 10 years after BVDV introduction in a (a) 400-head herd, (b) 100-head herd, and (c) 50-head herd.**

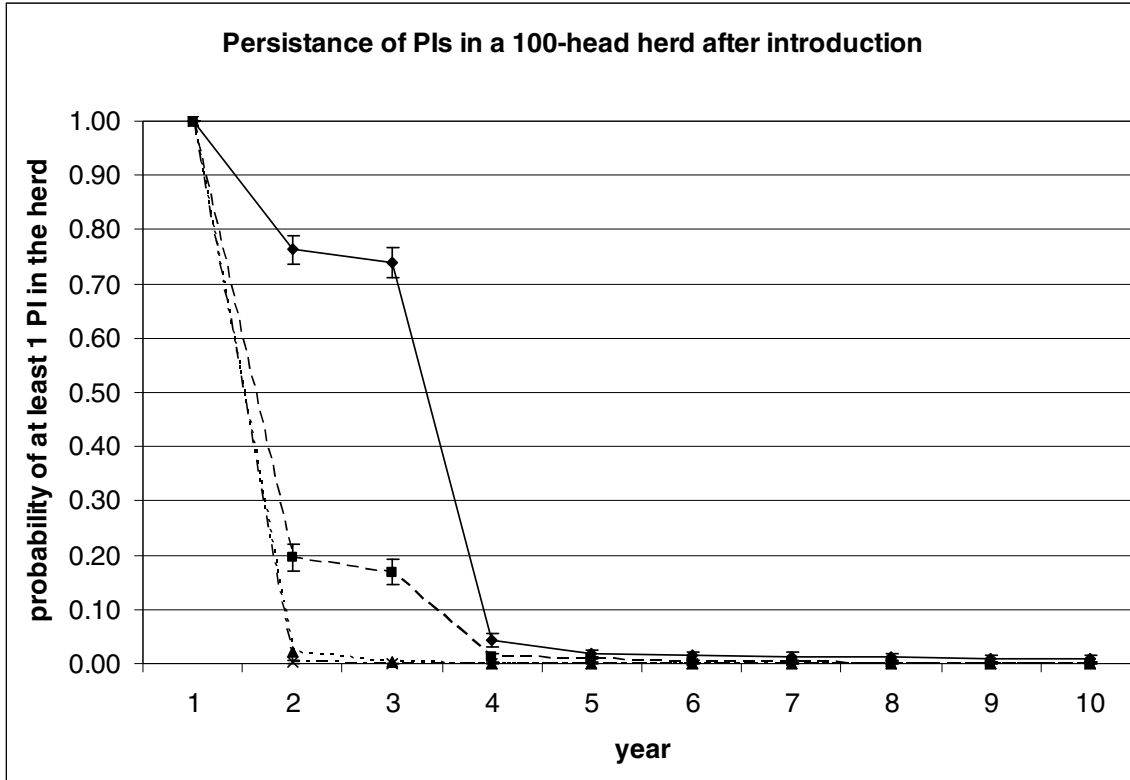
Key:



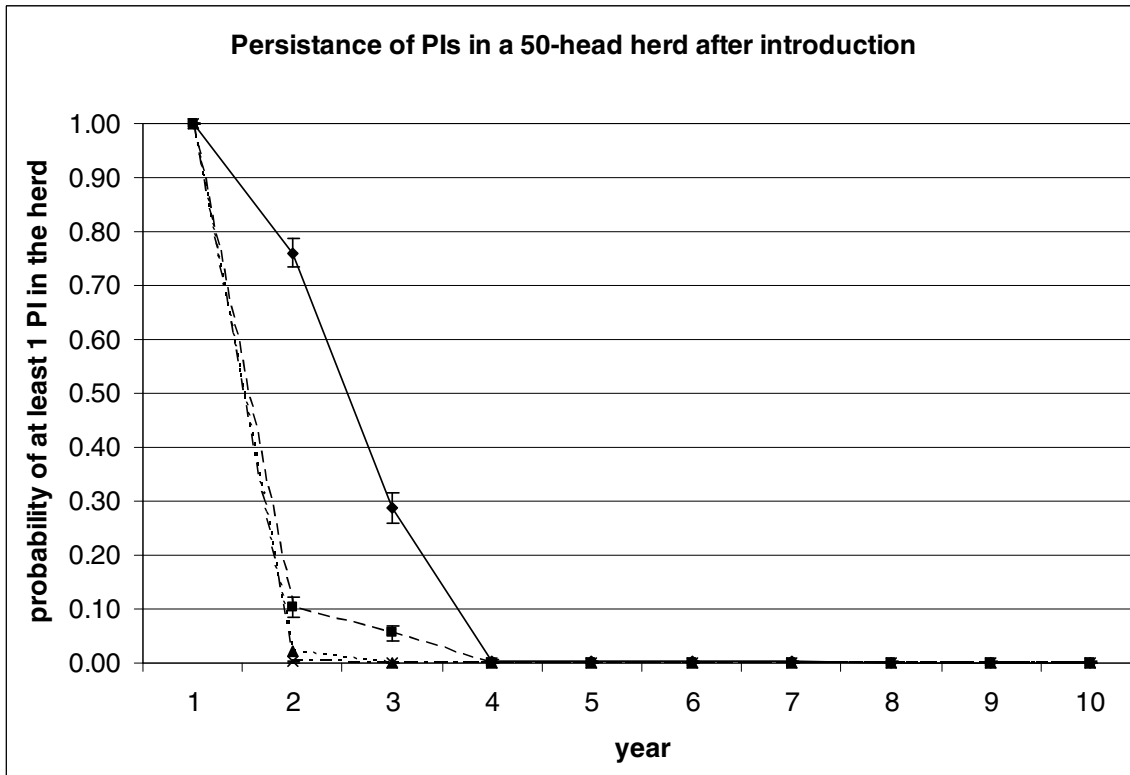
6(a)



6(b)



6(c)



In the current model, vaccination alone does decrease the probability of a longer outbreak when compared to no control program. The addition of vaccination to a pre-breeding test and cull of calves does not significantly decrease the probability of a longer outbreak compared to testing and culling calves alone, however. This model does not consider the possible transmission of virus by transiently infected animals, which would likely decrease the impact of testing and increase the value of vaccination.

Herd size has an effect on the duration of outbreaks. In the 400 head herd some probability of at least one PI remaining in the herd persists through 10 years is unless a test-and-cull strategy is utilized; in 50- and 100-head herds, self-clearance of BVDV infection was always observed within 8 and 10 years of introduction, respectively. This is, again, likely a result of the number of susceptible animals available in the herd for maintenance of infections. In a 50- or 100-head herd, it is possible for  $R_E$  to be lowered sufficiently by natural herd immunity, following infection, for self-clearance. 400-head herds are likely to have clusters of infections, maintaining clusters of susceptible animals that can be infected in following years, because the larger number of animals precludes complete mixing every year. This allows for maintenance of a rotating susceptible population of dams that can provide new PI calves to the herd each year. In addition, the total number of PIs in a 400-head herd is larger, so the probability that at least one will survive to infect a dam during the risk period is greater than in a 50-head herd, which may only have a single PI at a time. If one PI dies before breeding begins in a 50- or 100-head herd, there is a good probability that the infection is cleared in that year. However, if a single PI dies before the risk period in a 400-head herd, there is a good probability that there are other PIs to maintain the infection.

The model predicts that vaccination will significantly increase the probability of early clearance compared to doing nothing, but it does not alter the maximum predicted length of an outbreak. In a 400-head herd that does not test calves,  $R_E$  is not lowered to the point of always clearing BVDV from the herd within 10 years, though the probability of clearing the infection from the herd gradually increases. A herd of this size would have a supply of susceptible animals to maintain the infection if PIs remain in the herd during the risk period, even with vaccination, as vaccine efficacy is not always sufficient to create herd immunity. Also, as noted above, the larger number of PI calves maintains infection pressure, despite a high mortality rate, if a test-and-cull program is not implemented. These results are useful for decision making at the

herd management level, as they indicate that vaccination is not as effective in decreasing the length of an outbreak in 400-head herds as testing and culling calves pre-breeding, which is always an effective measure to ensure clearance of BVDV.

The model presented here does not take into account a constant source of infection; it is assumed that the herd is completely closed for 10 years following introduction. The effectiveness of the different control strategies might be changed by a constant source of BVDV introduction. The repeated importation of PIs would change clearance rates, but that could be controlled to some extent by testing imports. In the presence of other herds on shared fencelines or communal pasture, however, removing the source of the infection is not possible. In that situation, it may be necessary to vaccinate in order to control risk.

# **CHAPTER 5 - Economic risk analysis model for bovine viral diarrhea virus biosecurity in cow-calf herds**

## **Abstract**

A stochastic model was designed to calculate the cost-effectiveness of biosecurity strategies for Bovine Viral Diarrhea Virus (BVDV) in cow-calf herds. Possible sources of introduction considered were imported animals, including the calves of pregnant imports, and fence-line contact with infected herds, including stocker cattle raised in adjacent pasture. Spread of BVDV through the herd was modeled with a stochastic SIR model. Financial consequences of BVDV, including lost income, treatment costs, and the cost of biosecurity strategies, were calculated for 10 years, based on the risks of an open herd with a user-defined import profile. Results indicate that importing pregnant animals and stockers increase the financial risk of BVDV. Strategic testing is the most cost-effective biosecurity option when stockers are not imported; in most cases, vaccination in combination with testing is a co-dominant strategy for lowest risk to herds that import stockers. The choice of a biosecurity strategy is specific to the risks of any particular herd.

## **Introduction**

Bovine viral diarrhea virus (BVDV) costs the beef industry through decreased production and increased expenses (Wittum et al., 1994; Gunn et al., 1998; Bennett et al., 1999; Larson et al., 2002). It is a common disease in the US cattle herd (Houe et al., 1995b; Paisley et al., 1996; Chase et al., 2003).

Fetal infection between 40 and 125 days gestation leads to a persistently infected animal (PI) (Stokstad and Loken, 2002), which will shed virus for life through oculonasal discharges (Confer et al., 2005). Persistently infected animals are generally considered to be the primary source of BVDV introduction to a herd (Houe, 1999; Niskanen et al., 2002b).

Animals with transient infections (TIs), or infections of animals not in the risk period to become PI, experience a range of effects. In adults, these are mostly reproductive disorders such as abortion (Fredriksen et al., 1998), decreased conception rate (Houe and Meyling, 1991a;

McGowan et al., 1993a; McGowan et al., 1993b; Larsson et al., 1994; Wittum et al., 2001), early embryonic death (EED) (McGowan et al., 1993a; McGowan et al., 1993b), and congenital defects (Munoz-Zanzi et al., 2003; Ellsworth et al., 2006). In calves, common symptoms include immunosuppression leading to increased overall disease incidence (Castrucci et al., 1992; Bjorkman et al., 2000; Kozasaa et al., 2005).

Biosecurity against BVDV introduction includes testing imported animals and avoiding potentially infectious contact with infected herds, especially PI animals, as well as vaccination and test-and-cull programs. Testing strategies on imported animals aim to reduce the number of PIs introduced to a herd. Disease modeling of biosecurity programs based on herd testing protocols indicates that they may be cost-effectiveness (Stott et al., 2003). Vaccination is meant to decrease the spread of the virus once it is introduced to the herd; primarily, it is meant to prevent the birth of new PIs. Test-and-cull programs, often focusing on calves, are used to decrease the number of PIs present in the herd. This is commonly done before the breeding season, so as to decrease the number of sources of infection present during the risk period for producing more PIs. In addition to these strategies, avoiding the risk factors of herd contact on fencelines and communal pastures (Valle et al., 1999) also may prevent herd infection.

There are a multitude of stochastic models to study the effects of BVDV control programs on dairy herds (Innocent et al., 1997a; Innocent et al., 1997b; Cherry et al., 1998; Viet et al., 2004a; Viet et al., 2004b; Viet et al., 2005; Viet et al., 2006; Ezanno et al., 2007). Management differences between dairy and beef operations however, make those models less helpful in decision making for cow-calf producers. In particular, the limited breeding season of beef herds limits the risk period for BVDV infection leading to the birth of PI animals. This limited breeding season also increases the risk to the herd during this time period, as a greater proportion of dams will be in the risk period at one time than on a dairy, when breeding is usually spread out over the course of a year. Also, testing strategies in dairy herds often include monitoring of the bulk milk tank, which is not possible in beef herds. In addition, the spread of the virus is affected by the lower animal density of pastured beef herds compared to intensively managed dairies and by the continued contact between calves and adults until weaning in beef herds.

One model has been developed for BVDV in a cow-calf herd (Cleveland, 2003). This model was designed to examine the effect of test-and-cull strategies in an endemically infected



cow-calf herd. While this model is quite useful for the closed, infected herd looking to control the infection, it does not address the effectiveness of biosecurity strategies in herds not currently infected. It also does not provide estimates of cost-effectiveness, the level at which producers need to make decisions. One partial budget analysis examined the efficacy of testing for BVDV in incoming feedlot calves (Larson et al., 2005), but management factors again make the results less applicable to cow-calf operations. No models are available in the literature that address the overall impact of BVDV within cow-calf herds. The purpose of this study was to develop a stochastic risk-analysis model for the introduction, spread, cost, and control of BVDV in cow-calf herds.

## **Model**

This paper describes the integration of three Monte Carlo simulation models: a model for the annual introduction risk for BVDV to a cow-calf herd and the impact of biosecurity strategies, a model for the effects of BVDV for 10 years after introduction to a naïve cow-calf herd and the impact of control strategies, and a model for the economic costs of BVDV effects, biosecurity, and control for 10 years. The first two models have been described in previous chapters.

Briefly, the introduction model is a Monte Carlo model in which the probability of introducing BVDV to a herd in any year is calculated based on two risk categories, imports and fenceline contact. The number of PIs imported to the herd is based on the number of animals imported of a given age category and/or pregnancy status, the PI prevalence in that age group, including fetal prevalence for calves of pregnant imports, and the testing strategy to prevent PI importation. In addition, the probability of infection through contact with other herds through fenceline contact is modeled based in part on the prevalence of BVDV-infected herds, leading to a binomial variable for infection from fenceline contact. Fenceline infection can also be due to contact with imported stockers, or young animals grazed on available pasture for sale to feedlots or finishers. These values (number of infected PIs, fenceline infection, and communal pasture infection) are calculated independently for 10 years based on the import profile and management of the herd.

The model for the spread and effects of BVDV is driven by the introduction of the virus via the introduction model. Persistently infected animals are modeled to join the herd at the

beginning of the calving season in the year in which they are introduced. Infection in the herd is tracked by means of a modified Reed-Frost model based on the number of animals in the herd, the number of PIs in the herd, and the number of susceptible animals. This calculation is based on 3-week periods, for which conception is also modeled, allowing calculation of infections during the risk period for fetal persistent infection. The occurrence of fenceline infection is modeled as a binomial distribution based on prevalence of positive herds and estimated likelihood of transmission given a positive herd. When infection occurs by fenceline contact a single PI is added to the Reed-Frost calculation for a single 3-week time period.

The current model integrates both the presence of PIs from outside introduction and from endemic infections. In each year, the number of PIs produced in the previous year's breeding season are added to the herd during the corresponding 3-week period in the calving season. Persistently infected calves may be removed from the herd each year by a pre-breeding test-and-cull strategy.

The effects of BVDV were modeled primarily on an annual basis, based on the number of infections in each risk group. The number of abortions was based on the number of infected females on an annual basis, while the number of TI morbidities and mortalities were based on the number of infected calves on an annual basis. The number of PI mortalities was calculated for each 3-week period, allowing the PIs to be removed from the herd at death. The number of PI morbidities, however, was based on the number of PIs present on an annual basis, as the morbidities do not impact the risk to the herd. The number of EEDs and congenital defects, however, were calculated based on the number of infections during their respective risk periods, as are the number of PIs to be born. EEDs occurring before the end of the breeding season, at which time the dams are allowed to rebreed, were distinguished from EEDs occurring after the breeding season has ended and rebreeding was no longer possible.

Vaccination was modeled for breeding females on an annual basis, removing a number from the susceptible category for a single year based on vaccine efficacy. Vaccinated or otherwise immune animals give birth to immune calves. Immune calves remain so until weaning.

The economic model for the total 10-year cost of BVDV in cow-calf herds was based on a partial budget, integrating the costs of management and lost income. Parameters used in this model are shown in Table 5.1. Annual inputs from the introduction risk and herd spread models

were used to calculate the cost of BVDV for that year. Cost was based on both treatment cost, based on the number of calf morbidities, and lost income. Lost income was based on the price per pound, which was modeled on a 10-year draw of historical prices for the month of weaning, of the weight of animals not available for sale because of BVDV effects. Total lost income was calculated as the price per pound times the lost animal weight. The lost animal weight was the sum of the decrease in weaning weight in morbid calves and the total weight of calves lost to BVDV mortality. Decreased weaning weight was the difference in weaning weight from the average in calves that wean at a decreased weight because of BVDV. The decreased weaning weight of calves due to morbidity was modeled based on the number of morbid calves. Decreased weaning weights were also calculated for calves based on the number of EEDs occurring in cows that successfully rebred before the end of the breeding season. Calf mortality was modeled based on the number of abortions, EEDs occurring to cows that fail to rebreed, congenital defects, TI mortality, and PI mortality. The possible weaning weight of lost calves was based on a binomial calculation for the numbers of heifers and steers that were lost and on the weaning weight distribution for each gender.

**Table 5.1: Distributions used in economic analysis of biosecurity schemes**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Reference(s)</b>
Baby calf and fetal prevalence	Normal(0.59%,0.08%), Truncate(0,1)	(Caldow et al., 1993; Wittum et al., 2001; Cleveland, 2003)
Youngstock prevalence (stockers, bulls)	Normal(0.47%, 0.11%) Truncate (0, 1)	(Howard et al., 1990; Taylor et al., 1995; Fulton et al., 2000; Cleveland, 2003; Givens et al., 2003b; Loneragen et al., 2005; Gnad et al., 2005)
Heifer prevalence	If source herd is positive (from a binomial based on herd prevalence), youngstock prevalence/herd prevalence	Expert survey
Cow prevalence	Normal (0.07%, 0.04%) Truncate (0, 1)	(Smith et al., 2007)
Herd prevalence	Normal (10.16%, 2.7%) Truncate (0, 1)	(Wittum et al., 1997; Wittum et al., 2001)
Probability of fence-line infection	Pert (6%,47%,83%)	Expert survey
R <sub>0</sub> for PIs	Pert (5,7,12)	Personal opinion
R <sub>0</sub> from fence-line contact	Pert (1.2,3,5)	Personal opinion
Test sensitivity	Normal (97%, 1.6%) Truncate (0, 1)	(Frey et al., 1991; Mignon et al., 1992; Haines et al., 1992; Ellis et al., 1995; Sandvik and Krogsrud, 1995; Brinkhof et al., 1996; Deregt and Prins, 1998; Graham et al., 1998a; Schreiber et al., 1999; Saliki et al., 2000; Plavsic and Prodafikas, 2001; Grooms and Keilen, 2002; Deregt et al., 2002; Ozkul et al., 2002; Kim and Dubovi, 2003; Cornish et al., 2005; Walz et al., 2005; Kuhne et al., 2005; Kennedy et al., 2006)

**Table 5.1 (cont.)**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Reference(s)</b>
Abortion Rate	Pert (1.7%,10%,25%)	(Fredriksen et al., 1998; Hassig and Lubsen, 1998)
TI Mortality Rate (the proportion of all TI calves that will die in one year due to BVDV)	Pert (1%,5%,52%)	Expert survey
TI Case Fatality Rate (the proportion of morbid TI calves that will die in one year due to BVDV)	Pert (1%,7%,15%)	Expert survey
TI Morbidity Rate (the proportion of TI calves that will become morbid due to BVDV)	Mortality Rate/Case Fatality Rate (or 100%, if mortality rate is higher than case fatality rate)	Expert survey
Weight lost by morbidity (kg)	Normal (15.9, 3.5) Truncate (0, )	(Wittum et al., 1994)
PI Mortality Rate (the proportion of all PI calves that will die in one year due to BVDV)	Pert (10%,33%,100%)	Expert survey
PI Case Fatality Rate (the proportion of morbid PI calves that will die in one year due to BVDV)	Pert (40%,75%,100%)	Expert survey
PI Morbidity Rate (the proportion of TI calves that will become morbid due to BVDV)	Mortality Rate/Case Fatality Rate (or 100%, if mortality rate is higher than case fatality rate)	Expert survey
Probability of a PI fetus due to infection during the risk period (vertical transmission risk)	Normal (81%, 0.7%) Truncate (0, 1)	(Stokstad and Loken, 2002)

**Table 5.1 (cont.)**

<b>Parameter Description</b>	<b>Distribution</b>	<b>Reference(s)</b>
Probability of a deformed calf due to infection during the risk period	Pert (3.2%,13.6%,30%)	Expert survey
Duration of immunity from transient infection	50% for 1 year, 50% for 2 years	Personal opinion
Weaning weight (steers)	Normal (600,10) for 60 day breeding seasons Normal (580,10) for 100 day breeding seasons	
Weaning weight (heifers)	Normal (590,10) for 60 day breeding seasons Normal (570,10) for 100 day breeding seasons	
Cost of test (/head)	Pert (2.5,4,6)	Expert survey
Cost of vaccination (/head)	Uniform (0.75,1.5)	Expert survey
Cost of labor (\$/hour)	Pert (6,8,10)	Expert survey
Treatment costs (/calf)	Pert (4,10,15)	Expert survey

## ***1. Sensitivity analysis***

### ***1.1 Materials and Methods***

Sensitivity analysis was performed on the model to determine the importance of every distribution in Table 5.1. Sensitivity analysis on the introduction and the spread models have been reported (chapters 3 and 4). The integrated economic model reported here was analyzed with three combinations of the herd import profiles shown in Table 5.2 and the biosecurity strategies in Table 5.3. The first herd is A1, a 400-head herd importing 60 non-pregnant heifers, 4 bulls, 4 calves, and 100 stockers, using no biosecurity or control program (strategy M). The second herd is B3, a 100-head herd importing 15 pregnant heifers, 1 bull, and 100 stockers, vaccinating all breeding animals and testing all imported animals, including stockers and the calves of pregnant imports, and testing all calves pre-breeding (strategy Z). The third herd is C4,

a 50-head herd importing 8 pregnant heifers and one bull every other year, testing all imports, calves of pregnant imports, and all calves pre-breeding (strategy S). These three herds represent a mixed selection of the herds presented in the model results.

**Table 5.2: Herd import profiles used in model analysis.**

<b>Herd</b>	<b>number of breeding females</b>	<b>number of heifers imported</b>	<b>number of bulls imported</b>	<b>number of calves imported</b>	<b>number of stockers imported</b>
<b>A1</b>	400	60	4	4	100
<b>A2</b>	400	60	4	4	0
<b>A3</b>	400	60	4	0	100
<b>A4</b>	400	60	4	0	0
<b>B1</b>	100	15	1	1	100
<b>B2</b>	100	15	1	1	0
<b>B3</b>	100	15	1	0	100
<b>B4</b>	100	15	1	0	0
<b>C1</b>	50	8	1 every other year	1	100
<b>C2</b>	50	8	1 every other year	1	0
<b>C3</b>	50	8	1 every other year	0	100
<b>C4</b>	50	8	1 every other year	0	0

**Table 5.3: Biosecurity and control strategies used in model analysis; all animals in the testing category were tested.**

Strategy	Vaccination of breeding animals	Test Imported Adults*	Test Imported Calves and Calves of Pregnant Imports	Test all calves before breeding	Test Imported Stockers
M					
N	X				
O		X			
P			X		
Q				X	
R					X
S		X	X	X	
T		X	X	X	X
U	X	X			
V	X		X		
W	X			X	
X	X				X
Y	X	X	X	X	
Z	X	X	X	X	X

\*adults refers to breeding animals: heifers, cows, and bulls

Sensitivity of the total 10-year cost of BVDV to each herd was modeled by fixing each of the distributions listed in Table 5.4 individually over 2 values (low and high), as listed. The low value was the mean minus 2 standard deviations (S.D.) for normal distributions and the minimum value for pert distributions. The high value was the mean plus 2 S.D. for normal distributions and the maximum value for pert distributions. This allowed the sensitivity analysis to determine the impact of each distribution within approximately 95-100% of its expected range. Each simulation was run for 3000 iterations with a fixed number seed. The mean cost at each level of each distribution was calculated in @Risk. Differences between the low and high values for cost were calculated.



**Table 5.4: Comparison in the 10-year cost of BVDV in three herds when each input distribution is fixed at the mean minus 2 standard deviations and at the mean plus 2 standard deviations or at the minimum and maximum value.**

Input Distribution	Change in Input Distribution	Difference in the 10-year cost of BVDV with change in the input distributions		
		herd A1	herd B3	herd C4
		Heifers not pregnant	Heifers pregnant	Heifers pregnant
		Strategy M	Strategy Z	Strategy S
Abortion Rate	1.7%-25%	\$14,901.14	\$ 90.40	\$ 34.55
Calf prevalence	0.43%-0.77%	\$ 1,965.98	\$ 26.18	\$ 24.67
EED rate	0%-32%	\$ 330.62	\$ 0	\$ 0.28
Heifer weaning weight (lb)	570-610	\$ 620.71	\$ 2.57	\$ 1.17
Herd prevalence	4.8%-15.6%	\$10,417.32	\$ 1.80	\$ 0.62
Labor costs (\$/hour)	6-10	-	\$ 210.61	\$ 38.74
PI case fatality rate	40%-100%	\$ 0	\$ 0	\$ 0
PI mortality rate	10%-100%	\$ (5,643.67)	\$ (17.03)	\$ (8.24)
Probability of fenceline infection	6%-83%	\$14,027.64	\$ 37.83	-
R <sub>0</sub>	5-12	\$ 3,770.69	\$ 55.23	\$ 31.26
Steer weaning weight (lb)	580-620	\$ 492.55	\$ 1.62	\$ 0.75
Test sensitivity	91.3%-97.7%	-	\$ (121.75)	\$ (68.69)
TI case fatality rate	1%-15%	\$ (4,146.14)	\$ (11.56)	\$ (6.13)
TI Mortality Rate	1%-52%	\$92,267.71	\$ 225.36	\$ 129.38
Treatment costs/morbid calf (\$)	4-15	\$ 2,570.65	\$ 6.22	\$ 3.72
Unit cost of test (\$)	2.5-6	-	\$7,339.97	\$ 1,993.92
Unit cost of vaccination (\$)	0.75-1.50	-	\$ 750.00	-
Vaccine Efficacy	42%-100%	-	\$ (471.14)	-
Vertical transmission rate	79.6%-82.4%	\$ 417.21	\$ 0.59	\$ 0
Weight lost by Morbidity (kg)	8.9-22.9	\$ 7,166.89	\$ 17.29	\$ 10.33
Youngstock prevalence	0.25%-0.69%	\$12,498.16	\$ 50.48	\$ 24.51

## ***1.2 Results***

The difference in mean cost caused by changing each distribution from the low value to the high value is listed for the three herds in Table 5.4. In each of the herds examined, TI mortality was one of the three most influential distributions, but the relative effect was much higher in the 400-head herd than in the other two herds. The cost of an individual test was very influential inputs when animals were tested (strategies S and Z), but the influence of test sensitivity was small in comparison. The cost of a vaccine and vaccine efficacy were almost equally influential when animals were vaccinated (strategy Z). When no biosecurity program was used (strategy M), herd prevalence and youngstock prevalence had a large effect on mean cost, but the prevalence distributions were less influential when testing was used (strategies S and Z). For all three herds, an increased PI mortality rate, the proportion of all PI calves that die, always decreased the mean cost of BVDV, but the PI case fatality rate, the proportion of PI calves with morbidity that die, had no impact on cost.

## ***2. Validation***

### ***2.1 Materials and Methods***

Validation of the model was performed using two published outbreaks in cow-calf herds in which the source of the virus could be determined.

The first (Taylor and Rodwell, 2001) describes the effects of BVDV for one year after the purchase of a 269 cows by an 877 cow herd. Morbidity, mortality, the number of PIs in the herd, and the number of lost pregnancies (due to EEDs and abortions) were tabulated and published. The model was given the input of 269 pregnant cows imported to an 877-head naïve herd.

The second outbreak (Campen et al., 2000) was in a 391-head herd that shared fenceline with 3 herds and for which a subset of the outbreak herd shared pasture with a number of herds. The number of mortalities and lost pregnancies were tabulated after one year, and a serosurvey of all calves was performed to identify the number of TI calves. This herd was modeled to have 391 breeding animals, with 3 other herds in fenceline contact, and 2 other herds in contact on communal pasture.

The simulation for each herd was run for 3000 iterations and the mean and standard deviation for each of the categories in the year of introduction was calculated in @Risk. Means

and 95% confidence intervals were calculated with the Student's t distribution. Sensitivity analysis was performed, as described above, for values in which the observed value was not contained in the 95% confidence interval.

## 2.4 Results

The comparison between observed and predicted values for each herd are presented in Table 5.5. For the herd that imported cows (Taylor and Rodwell, 2001), the observed values of all variables were within the range predicted, but the mean number of mortalities predicted was substantially less than the observed value and the mean number of lost pregnancies predicted was substantially higher than observed. Sensitivity analysis on total morbidities after one year indicated that a value as high as observed was most closely approached by the model mean if the reproductive rate,  $R_0$ , was fixed at its highest value, 12. Sensitivity analysis on the number of lost pregnancies indicated that the observed value was predicted by the model mean if the abortion rate was fixed at its 5<sup>th</sup> percentile, 1.7%. For the herd that shared both pasture and fenceline with other herds (Campen et al., 2000), the observed values of all variables were within the range predicted and mean predictions were similar to observed values.

**Table 5.5: Comparisons of observed and predicted values from reported BVDV outbreaks in naïve cow-calf herds**

Herd	output	Observed value	mean	Lower CI	Upper CI
(Taylor and Rodwell, 2001)	Morbidity	265	81.51	0	319.51
	Mortality	77	12.75	0	55.96
	Total PIs	48	22.24	0	77.86
	Lost pregnancies	13	78.55	0	163.47
(Campen et al., 2000)	Lost pregnancies	37	31.21	0	70.64
	Infected Calves	30	29.56	0	110.41
	Mortality	1	3.92	0	17.44

### ***3. Model Application***

#### ***3.1 Materials and Methods***

The model was run for 3000 iterations with a fixed random number seed for each of the herds listed in Table 5.2 with each of the appropriate biosecurity strategies listed in Table 3. This was a sufficient number of iterations for mean, standard deviation, and percentiles of all outputs in all simulations to converge within 5%. For each of the herds, separate simulations were run to model importation of pregnant heifers or importation of non-pregnant heifers. The mean and standard deviation for total 10-year cost was calculated in @Risk. The 95% confidence intervals around the mean were calculated with the Student's t distribution. Dominance graphs were built for each herd with all biosecurity strategies. In addition, the probability of exceeding a target value for 10-year cost of BVDV in the herd was calculated with each simulation. Calculated costs included the cost of disease and the cost of prevention and treatment for each simulation. The target values were set as \$40,000 for a 400-head herd, \$7,500 for a 100-head herd, and \$2,500 for a 50-head herd; these values were selected to represent the author's opinion of the average return to labor and management for each cow-calf herd size for one year. The 95% confidence intervals around the probabilities were calculated with the normal distribution Z statistic.

#### ***3.2 Results***

In none of the simulations were there differences between the mean costs of any biosecurity strategy (data not shown). First-order dominance was not observed for any herd or for any strategy. The results of the target analysis are presented in Table 5.6 for herds importing non-pregnant heifers and in Table 5.7 for herds importing pregnant heifers; dominant or co-dominant strategies are in bold type for each column. Cumulative distribution functions for 3 of the herds analyzed are shown in Figure 5.1.

**Table 5.6: Probability of exceeding target value<sup>1</sup> of costs due to BVDV over 10 years in a herd that imports non-pregnant heifers**

5.6(a)

strategy	A1	A2	A3	A4
M	45% <sup>a</sup> (42-48%)	35% <sup>a</sup> (32-38%)	40% <sup>a</sup> (37-43%)	30% <sup>a</sup> (27-32%)
N	19% <sup>bc</sup> (17-22%)	17% <sup>b</sup> (15-20%)	17% <sup>b</sup> (14-19%)	15% <sup>b</sup> (13-18%)
O	27% <sup>d</sup> (24-29%)	13% <sup>b</sup> (11-16%)	19% <sup>bc</sup> (17-22%)	5% <sup>c</sup> (4-7%)
P	41% <sup>a</sup> (38-44%)	30% <sup>a</sup> (27-33%)		
Q	43% <sup>a</sup> (40-46%)	36% <sup>a</sup> (33-39%)	42% <sup>d</sup> (39-45%)	35% <sup>d</sup> (32-38%)
R	40% <sup>e</sup> (37-43%)		34% <sup>e</sup> (31-37%)	
S	16% <sup>b</sup> (14-18%)	5% <sup>c</sup> (4-7%)	16% <sup>b</sup> (14-18%)	5% <sup>c</sup> (4-7%)
T	<b>7%<sup>e</sup> (5-9%)</b>		<b>7%<sup>f</sup> (5-9%)</b>	
U	<b>6%<sup>e</sup> (4-7%)</b>	4% <sup>c</sup> (3-5%)	<b>4%<sup>f</sup> (2-5%)</b>	<b>2%<sup>e</sup> (1-3%)</b>
V	17% <sup>b</sup> (15-20%)	16% <sup>b</sup> (13-18%)		
W	25% <sup>cd</sup> (22-28%)	24% <sup>d</sup> (21-26%)	23% <sup>c</sup> (21-26%)	22% <sup>f</sup> (20-25%)
X	22% <sup>bc</sup> (19-24%)		19% <sup>bc</sup> (17-22%)	
Y	<b>4%<sup>e</sup> (3-5%)</b>	<b>2%<sup>e</sup> (1-3%)</b>	<b>4%<sup>f</sup> (3-5%)</b>	<b>2%<sup>e</sup> (1-3%)</b>
Z	<b>3%<sup>e</sup> (2-5%)</b>		<b>3%<sup>f</sup> (2-5%)</b>	

<sup>1</sup>Target values: Herd A=\$40,000

a,b,c,d,e,f,g Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**

## 5.6(b)

strategy	B1	B2	B3	B4
M	38% <sup>a</sup> (35-41%)	22% <sup>a</sup> (20-25%)	37% <sup>a</sup> (34-40%)	20% <sup>a</sup> (18-22%)
N	15% <sup>b</sup> (13-17%)	12% <sup>b</sup> (10-14%)	14% <sup>b</sup> (12-16%)	12% <sup>b</sup> (10-14%)
O	26% <sup>c</sup> (23-29%)	5% <sup>c</sup> (3-6%)	24% <sup>c</sup> (21-26%)	<b>2%<sup>c</sup> (1-2%)</b>
P	37% <sup>a</sup> (34-40%)	20% <sup>a</sup> (18-23%)		
Q	40% <sup>a</sup> (37-43%)	26% <sup>a</sup> (23-29%)	39% <sup>a</sup> (36-42%)	25% <sup>d</sup> (23-28%)
R	31% <sup>d</sup> (29-34%)		28% <sup>d</sup> (26-31%)	
S	23% <sup>c</sup> (21-26%)	<b>2%<sup>d</sup> (1-3%)</b>	23% <sup>c</sup> (21-26%)	<b>2%<sup>c</sup> (1-3%)</b>
T	100% <sup>e</sup>		100% <sup>e</sup>	
U	<b>5%<sup>f</sup> (3-6%)</b>	<b>2%<sup>d</sup> (1-3%)</b>	<b>4%<sup>f</sup> (2-5%)</b>	<b>1%<sup>c</sup> (0-1%)</b>
V	14% <sup>b</sup> (12-17%)	12% <sup>b</sup> (10-14%)		
W	26% <sup>c</sup> (24-29%)	23% <sup>a</sup> (20-26%)	26% <sup>c d</sup> (23-28%)	22% <sup>a d</sup> (20-25%)
X	27% <sup>c d</sup> (25-30%)		25% <sup>c d</sup> (22-28%)	
Y	8% <sup>g</sup> (6-9%)	<b>2%<sup>d</sup> (1-3%)</b>	7% <sup>g</sup> (6-9%)	<b>2%<sup>c</sup> (1-3%)</b>
Z	100% <sup>e</sup>		100% <sup>e</sup>	

<sup>1</sup>Target values: Herd B=\$7,500

a,b,c,d,e,f,g Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**

## 5.6(c)

strategy	C1	C2	C3	C4
M	43% <sup>a</sup> (40-46%)	17% <sup>a</sup> (14-19%)	41% <sup>a</sup> (38-44%)	13% <sup>a</sup> (11-16%)
N	20% <sup>b</sup> (17-22%)	11% <sup>b</sup> (9-13%)	18% <sup>b</sup> (15-20%)	9% <sup>b</sup> (8-11%)
O	36% <sup>c</sup> (33-39%)	<b>5%<sup>c</sup> (4-7%)</b>	34% <sup>c</sup> (31-37%)	<b>1%<sup>c</sup> (1-2%)</b>
P	41% <sup>a,c</sup> (38-44%)	14% <sup>a,b</sup> (11-16%)		
Q	56% <sup>d</sup> (53-59%)	18% <sup>a</sup> (16-20%)	54% <sup>d</sup> (51-57%)	15% <sup>a</sup> (13-17%)
R	100% <sup>e</sup>		100% <sup>e</sup>	
S	67% <sup>f</sup> (64-70%)	23% <sup>d</sup> (21-26%)	67% <sup>f</sup> (64-70%)	23% <sup>d</sup> (20-26%)
T	100% <sup>e</sup>		100% <sup>e</sup>	
U	<b>14%<sup>g</sup> (12-16%)</b>	<b>4%<sup>c</sup> (3-5%)</b>	<b>11%<sup>g</sup> (9-13%)</b>	<b>1%<sup>c</sup> (0-2%)</b>
V	18% <sup>b</sup> (16-20%)	10% <sup>b</sup> (8-11%)		
W	96% <sup>h</sup> (95-97%)	93% <sup>c</sup> (92-95%)	96% <sup>h</sup> (95-97%)	93% <sup>e</sup> (92-95%)
X	100% <sup>e</sup>		100% <sup>e</sup>	
Y	100% <sup>e</sup>	100% <sup>f</sup>	100% <sup>e</sup>	100% <sup>f</sup>
Z	100% <sup>e</sup>		100% <sup>e</sup>	

<sup>†</sup>Target values: Herd C=\$2,500

<sup>a,b,c,d,e,f,g</sup>Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**

**Table 5.7: Probability of exceeding target value<sup>1</sup> of costs due to BVDV over 10 years in a herd that imports pregnant heifers**

5.7(a)

strategy	A1	A2	A3	A4
M	74% <sup>a</sup> (71-77%)	70% <sup>a</sup> (67-73%)	73% <sup>a</sup> (70-76%)	69% <sup>a</sup> (66-71%)
N	44% <sup>bc</sup> (41-48%)	43% <sup>b</sup> (40-46%)	43% <sup>b</sup> (40-46%)	41% <sup>bc</sup> (38-45%)
O	75% <sup>a</sup> (72-77%)	71% <sup>a</sup> (68-74%)	74% <sup>a</sup> (71-76%)	69% <sup>a</sup> (67-72%)
P	43% <sup>b</sup> (40-46%)	33% <sup>c</sup> (30-36%)	42% <sup>b</sup> (39-45%)	32% <sup>d</sup> (29-35%)
Q	55% <sup>d</sup> (52-58%)	48% <sup>b</sup> (45-51%)	54% <sup>c</sup> (51-57%)	47% <sup>b</sup> (44-50%)
R	76% <sup>a</sup> (73-79%)		75% <sup>a</sup> (72-77%)	
S	17% <sup>e</sup> (15-20%)	7% <sup>d</sup> (5-8%)	17% <sup>d</sup> (15-20%)	7% <sup>e</sup> (5-8%)
T	9% <sup>f</sup> (7-10%)		9% <sup>e</sup> (7-10%)	
U	45% <sup>bc</sup> (42-48%)	43% <sup>b</sup> (40-46%)	43% <sup>b</sup> (39-46%)	41% <sup>bc</sup> (38-44%)
V	18% <sup>e</sup> (16-21%)	17% <sup>e</sup> (14-19%)	18% <sup>d</sup> (16-21%)	16% <sup>f</sup> (14-19%)
W	38% <sup>b</sup> (35-41%)	37% <sup>c</sup> (34-40%)	37% <sup>b</sup> (34-40%)	36% <sup>cd</sup> (33-39%)
X	50% <sup>c</sup> (47-53%)		48% <sup>b</sup> (45-51%)	
Y	<b>5%<sup>g</sup> (4-7%)</b>	<b>4%<sup>f</sup> (3-5%)</b>	<b>5%<sup>f</sup> (4-7%)</b>	<b>4%<sup>g</sup> (3-5%)</b>
Z	<b>5%<sup>g</sup> (4-7%)</b>		<b>5%<sup>f</sup> (4-7%)</b>	

<sup>1</sup>Target values: Herd A=\$40,000

a,b,c,d,e,f,g Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**



## 5.7(b)

strategy	B1	B2	B3	B4
M	57% <sup>ab</sup> (53-60%)	45% <sup>a</sup> (42-48%)	55% <sup>ab</sup> (52-58%)	43% <sup>a</sup> (40-46%)
N	27% <sup>c</sup> (25-30%)	24% <sup>bc</sup> (22-27%)	27% <sup>cd</sup> (24-29%)	23% <sup>bc</sup> (21-26%)
O	57% <sup>ab</sup> (54-60%)	45% <sup>a</sup> (42-48%)	56% <sup>ab</sup> (53-59%)	43% <sup>a</sup> (40-46%)
P	38% <sup>d</sup> (35-41%)	20% <sup>b</sup> (17-22%)	37% <sup>e</sup> (34-40%)	19% <sup>b</sup> (17-22%)
Q	48% <sup>e</sup> (45-51%)	32% <sup>d</sup> (29-35%)	47% <sup>f</sup> (44-50%)	31% <sup>d</sup> (28-34%)
R	59% <sup>a</sup> (56-62%)		57% <sup>a</sup> (54-60%)	
S	24% <sup>c</sup> (21-27%)	<b>3%<sup>e</sup> (2-4%)</b>	24% <sup>c</sup> (21-27%)	<b>3%<sup>e</sup> (2-4%)</b>
T	100% <sup>f</sup>		100% <sup>g</sup>	
U	28% <sup>c</sup> (25-30%)	23% <sup>bc</sup> (21-26%)	27% <sup>cd</sup> (24-29%)	23% <sup>bc</sup> (20-25%)
V	15% <sup>g</sup> (13-17%)	12% (10-14%)	15% <sup>h</sup> (13-17%)	12% <sup>f</sup> (10-14%)
W	32% <sup>c</sup> (29-35%)	27% <sup>cd</sup> (25-30%)	31% <sup>d</sup> (28-34%)	27% <sup>cd</sup> (24-29%)
X	52% <sup>bc</sup> (49-55%)		50% <sup>bf</sup> (47-53%)	
Y	<b>8%<sup>h</sup> (7-10%)</b>	<b>3%<sup>e</sup> (2-4%)</b>	<b>8%<sup>i</sup> (7-10%)</b>	<b>3%<sup>e</sup> (2-4%)</b>
Z	100% <sup>f</sup>		100% <sup>g</sup>	

<sup>i</sup>Target values: Herd B=\$7,500

a,b,c,d,e,f,g Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**

## 5.7(c)

strategy	C1	C2	C3	C4
M	57% <sup>a</sup> (54-60%)	38% <sup>a</sup> (35-41%)	56% <sup>a</sup> (53-59%)	35% <sup>a</sup> (32-38%)
N	33% <sup>b</sup> (30-36%)	25% <sup>b</sup> (23-28%)	32% <sup>b</sup> (29-35%)	24% <sup>b</sup> (21-27%)
O	58% <sup>a</sup> (55-61%)	37% <sup>a</sup> (34-40%)	57% <sup>a</sup> (54-60%)	34% <sup>a</sup> (31-37%)
P	43% <sup>c</sup> (40-46%)	<b>14%<sup>c</sup> (11-16%)</b>	43% <sup>c</sup> (39-46%)	<b>13%<sup>c</sup> (11-15%)</b>
Q	68% <sup>d</sup> (65-71%)	39% <sup>a</sup> (36-42%)	67% <sup>d</sup> (64-70%)	37% <sup>a</sup> (34-40%)
R	100% <sup>e</sup>		100% <sup>e</sup>	
S	68% <sup>d</sup> (65-71%)	25% <sup>b</sup> (22-28%)	68% <sup>d</sup> (65-71%)	25% <sup>b</sup> (22-27%)
T	100% <sup>e</sup>		100% <sup>e</sup>	
U	36% <sup>b</sup> (33-39%)	27% <sup>b</sup> (24-30%)	34% <sup>b</sup> (31-37%)	25% <sup>b</sup> (22-28%)
V	<b>20%<sup>f</sup> (18-23%)</b>	<b>11%<sup>c</sup> (9-12%)</b>	<b>20%<sup>f</sup> (17-22%)</b>	<b>10%<sup>c</sup> (8-12%)</b>
W	97% <sup>g</sup> (96-98%)	95% <sup>d</sup> (94-96%)	97% <sup>g</sup> (96-98%)	95% <sup>d</sup> (93-96%)
X	100% <sup>e</sup>		100% <sup>e</sup>	
Y	100% <sup>e</sup>	100% <sup>e</sup>	100% <sup>e</sup>	100% <sup>e</sup>
Z	100% <sup>e</sup>		100% <sup>e</sup>	

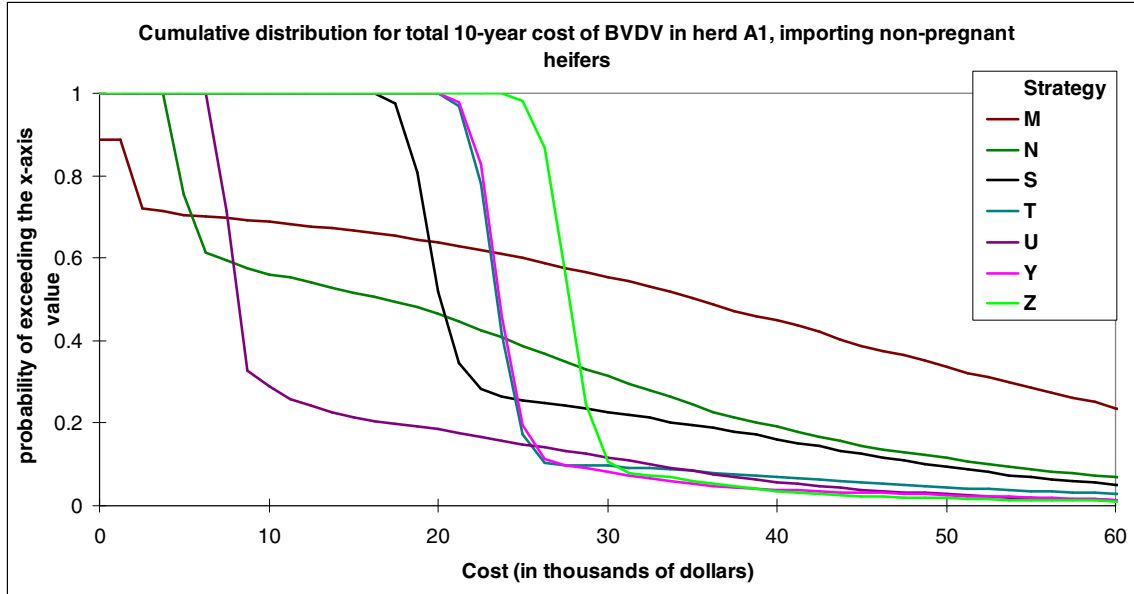
<sup>1</sup>Target values: Herd C=\$2,500

a,b,c,d,e,f,g Numbers within columns that share a superscript are not significantly different, based on overlapping confidence intervals

**Numbers in bold are the lowest risk option for that column (herd).**

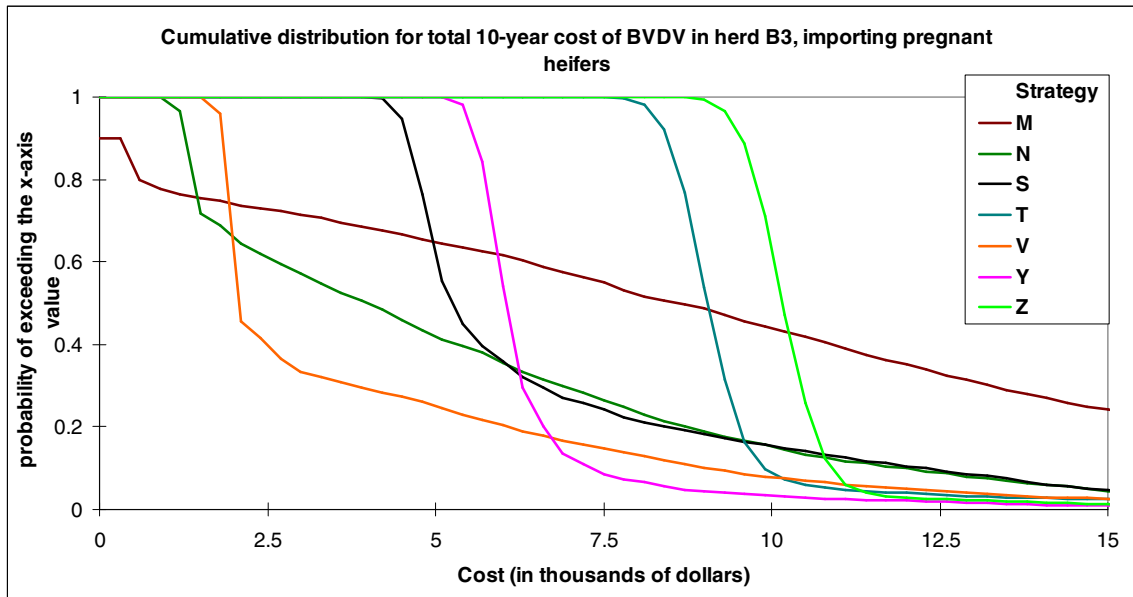
**Figure 5.1: Cumulative distribution functions for 10-year cost due to BVDV with selected biosecurity strategies.**

5.1(a): A 400-head herd importing 60 non-pregnant heifers, 4 bulls, 4 calves, and 100 stockers annually.



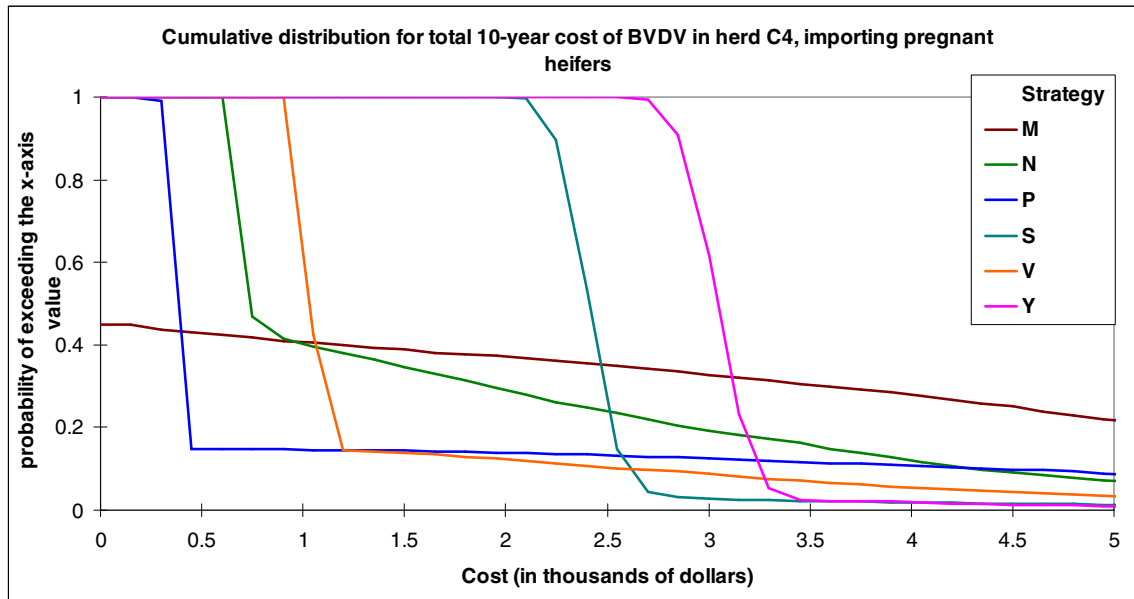
Strategy: M (do nothing), N (vaccinate breeding animals), S (test imported heifers, bulls, and calves and test and cull all calves in the herd pre-breeding), T (test all imports, including stockers, and test and cull all calves in the herd pre-breeding), U (vaccinate breeding animals and test imported heifers and bulls), Y (test as in S and vaccinate breeding animals), Z (test as in T and vaccinate breeding animals)

5.1(b): A 100-head herd importing 15 pregnant heifers, 1 bull, and 100 stockers annually.



Strategy: M (do nothing), N (vaccinate breeding animals), S (test imported heifers, bulls, calves, and calves of pregnant imports and test and cull all calves in the herd pre-breeding), T (test all imports, including stockers, and test and cull all calves in the herd pre-breeding), V (vaccinate breeding animals and test imported calves and calves of pregnant imports), Y (test as in S and vaccinate breeding animals), Z (test as in T and vaccinate breeding animals)

5.1(c): A 50-head herd importing 8 pregnant heifers annually and 1 bull every other year.



Strategy: M (do nothing), N (vaccinate breeding animals), P (test calves of pregnant imports), S (test imported heifers, bulls, and calves and test and cull all calves in the herd pre-breeding), V (vaccinate breeding animals and test calves of pregnant imports), Y (test as in S and vaccinate breeding animals)

## Discussion

The model presented here predicts the economic risks associated with specific management decisions as regards BVDV, including the importation of different classes of cattle and biosecurity strategies. While the model does not predict, in a deterministic sense, the most cost-effective strategy for BVDV, it does give herd-specific risk calculations that can assist in decision making allowing the individual producer to include their individual risk preferences into the decision making process. The outcome utilized is based on the probability of exceeding a target value of cost to account for both the costs of disease and prevention. This allows decision making based on the cost of disease and the effectiveness and cost of mitigation.

Validation of a stochastic disease model with field data is an accepted method (Cleveland, 2003; Viet et al., 2004a). However, field data is either available from endemically infected herds, for which this model is not designed, or from outbreaks, in which the source of

virus is not often known. Only two published outbreak reports were found to be sufficiently detailed for validation of this model, and a limited number of effects were recorded for each. With the observations available for each herd, the model was able to predict an outcome not significantly different from the observed value for all variables. In one validation (Taylor) we substantially underestimated the number of morbidities but the subsequent sensitivity analysis indicated that a high  $R_0$  resolved the difference. It seems reasonable that this outbreak may have experienced an elevated  $R_0$ , accounting for the increase in morbidities. It is known that the reproductive rate of a virus may vary with strain, animal density, nutritional status of the animals, and other management factors (Dohoo et al., 2003). It is quite possible that a publication bias towards larger outbreaks leads to outbreaks in the published literature being those in which the  $R_0$  was higher than average. In that case, the inability of the model to predict the outcome of the outbreak perfectly is to be expected. In the case of the number of lost pregnancies, in which the model over-predicted the mean compared to that reported by Taylor and Rodwell, sensitivity analysis indicated that a low-end value for the abortion rate (1.7%) would cause the model to predict the observed number of lost pregnancies. Abortion rate is also a distribution that may involve natural variability, so it is possible that the strain involved in the reported outbreak was less likely to cause abortions than other strains observed in the literature that provided the distribution. If the model is run with the  $R_0$  fixed at 12 and the abortion rate fixed at 1.7%, the discrepancy in the number of lost pregnancies decreases by 99% (from 78 to 12 predicted lost pregnancies, compared to the 13 observed) and the discrepancy between observed and predicted calf morbidity decreases by 10% (from 81 to 112 predicted morbidities, compared to 265 observed). However, given the large numbers of other transient infections in this outbreak (calf morbidity and mortality), it seems unlikely that this strain was less virulent than normal in regards to the abortion rate. Another possible explanation for the model's over-prediction is that the observed value from the outbreak is an estimate based on the difference between the herd's average calving rate and the calving rate after the outbreak. As this is an estimate it is possible that more pregnancies were lost due to BVDV than reported which would be more consistent with the model's predictions.

The sensitivity analysis of the integrated model gives some intriguing results (Table 4). The impact of the distributions used in the model indicates that it is the variability around the biological factors that are most influential in the model, and that most economic factors, such as

the cost of labor, the cost of treatment, and the weaning weight of heifers or steers, rarely impact the overall cost of BVDV. The exception is the cost of testing, which is the most influential distribution if animals are tested, and the cost of vaccination, which is influential when animals are vaccinated. When testing strategies are used, a \$2 increase in the cost of a test leads to a mean cost increase of \$4,193 in a 100-head herd testing 131 imports and close to 100 calves every year, while the same increase leads to a mean cost increase of \$1,139 in a 50-head herd testing 17 imports and close to 50 calves every year; this is 0.6% and 0.5% of expected return to labor and management over 10 years, respectively. The impact of increasing the cost of vaccine by \$0.75 was only one-fifth as large, but that is likely due in part to the fact that many more animals were tested than were vaccinated with the biosecurity strategy used. These findings would indicate that the cost of the test and the vaccine would have a substantial impact on the choice of biosecurity strategy. The cost of the vaccine is not correlated with the vaccine efficacy, which is almost an equally influential variable; a more expensive vaccine that is more efficacious could be cost-effective. Vaccine efficacy is also a distribution incorporating substantial natural variability. Published estimates of vaccine efficacy in preventing PIs vary considerably. Some of this variability may be due to differences between vaccines but some also may be due to differences in the specifics of the trials related to cattle factors and viral challenge factors. In a production setting, additional variability exists due to vaccine handling and management of cattle that may affect their ability to mount an effective immune response. The model attempts to capture some of the variation seen with different vaccines, their performance against different strains of BVDV, and the management factors that affect their efficacy.

The biological variable shown to be most influential is the TI mortality rate. This distribution is particularly wide, but it is a pert distribution with a long tail to the right, so the maximum value used in the sensitivity analysis would rarely be used. Surveyed experts believed based on their personal experience in cow-calf herds experiencing outbreaks that while TI mortality rates were generally modest, in some cases high mortality rates were observed. This phenomenon may be due to variability seen in BVDV strains, so these distributions are appropriately wide and influential in the model. The distribution was more influential when no control strategy was used (herd A1); this herd would experience more TIs over 10 years, which would explain the greater influence of the TI mortality rate. Herd prevalence and youngstock prevalence were also very influential if no testing strategy was used. The influence of

youngstock prevalence is likely due to the large number of youngstock imports (stockers, heifers, and bulls) in herds modeled here, while increased herd prevalence would decrease the clustering of PI heifers, allowing for a more constant introduction of virus to maintain high infection levels in the herd. If animals were tested, the effect of prevalence values is substantially decreased.

The sensitivity analysis also showed that an increase in the PI mortality rate decreased the mean cost of BVDV for all herds scenarios examined. In contrast, variation in the PI case fatality rate, which is used to calculate the PI morbidity rate, never impacted the mean cost. Anecdotal evidence has long suggested that the best thing a PI calf could do, in a cow-calf herd or in a feedlot, would be to die young, thus removing itself as a source of infection and preventing ongoing costs associated with feed and morbidity. This finding supports that view; as PI mortality is calculated every three weeks within the SIR model, a high mortality rate causes the PIs to be removed from the model quickly, protecting the other animals in the herd from transient infections. In comparison, the relatively small numbers of PI calves prevents PI morbidities, which are calculate via the PI case fatality rate, to have a large effect on total cost compared to TI morbidities.

The predictions of the model with regards to cost show no significant differences between biosecurity strategies in mean cost of BVDV over 10 years and no first-order dominance in descending probability cost distributions, regardless of the herd modeled. This is related to the low prevalence of PIs resulting in introduction of a PI being a rare event. With a rare outcome, the mean cost of the disease is skewed to the left, obscuring differences in control programs. This was the primary reason to build a stochastic model for BVDV, so these results are not unexpected.

When mean differences are not significantly different, and when first-order dominance does not occur, decisions must be made based on other criteria. The results presented here, based on the probability of exceeding a target value, are only one method of risk-based decision making. Target analysis is an intuitive method for decision making in cow-calf production enterprises.

It can be seen, looking at Tables 6 and 7, that herd size and import profile are important determinants in the risk of exceeding the target cost. Comparing tables 6 and 7, herds importing pregnant heifers have a significantly higher probability of exceeding the target cost than herds importing non-pregnant heifers if no biosecurity strategy is employed (strategy M). Testing



adult imports (heifers and bulls) only (strategy O) decreases risk compared to doing nothing (strategy M) only in herds importing non-pregnant heifers, and is only co-dominant in the 50-head herds importing non-pregnant heifers and no stockers (herds C2 and C4) or the 100-head herds importing only non-pregnant adults (heifers and bulls, herd B4). Pregnant heifers may be PI and carrying a PI calf, but alternately they may have been transiently infected during the risk period and be carrying a PI calf. A testing strategy that only tests the replacement heifers would miss the PI calf of a previously TI heifer and allow introduction of BVDV to the herd. Conversely testing the calves of pregnant imports after birth and before breeding season will allow detection of the calf and identification of the dam for further testing. In this model, the dams of positive calves are not tested unless all the replacement heifers are tested, but the calves of positive dams are automatically removed. Calves and fetal imports, are a proportionately greater risk to the herd than their dams because the risk associated with imports increases as the age of the imports decreases, due to higher PI prevalence in younger animals.

Regardless of the other import decisions, importing stockers increases the risk of exceeding the target cost. This assumes there is fence-line contact between the breeding herd and the stockers. In U.S. beef production systems, stockers may be imported at relatively high rates into a breeding herd to take advantage of additional grazing or as a market risk management option. The number of stockers modeled here represents approximately 1 truckload of stockers and is meant to be representative of the lower end of potential import rates. Vaccinating breeding animals and testing adult imports, including stockers (strategy U), is always one of the lowest risk strategies if stockers and non-pregnant heifers are imported. If imported heifers are pregnant, however, a combination of vaccinating and testing the imported calves and calves of pregnant imports (strategy V) is dominant for 50-head herds, while for 100-head herds a combination of vaccination, testing all imports except stockers, and testing and culling calves pre-breeding is dominant (strategy Y). In the 400-head herd importing stockers and pregnant heifers, co-dominance is observed between strategy Y and strategy Z, which adds the testing of imported stockers. Testing the imported stockers decreases the risk of importing PI stockers, but the cost of testing stockers is greater than the target value for 50-head herds and the cost of combining stoker testing with the other testing strategies (strategies T and Z) is greater than the target value for 100-head herds. Because the base cost of testing stockers is so high, it is only among the lowest-risk options for 400-head herds, where it is co-dominant with less expensive

strategies. If management allows, the most cost effective management of the risk due to stockers may be to assure that there is no contact between the imported stockers and the breeding herd, which would be comparable to the herds in these results that did not import stockers.

In the absence of stockers, vaccination of breeding animals as a single biosecurity practice is only necessary to decrease risk in the 400-head herd, although it is among the co-dominant options for all herds, and vaccination alone decreases risk compared to doing nothing. Instead, testing without vaccination becomes co-dominant. When imported heifers are not pregnant and few or no calves are imported, testing only adults (strategy O) is the lowest-cost co-dominant option for 50- and 100-head herds. If pregnant heifers are imported, however, both 50- and 100- head herds require testing of imported calves, alone or in combination with other strategies. Based on the results of this model, it would be advisable for smaller herds whose risk is based on importation of animals to the breeding herd, rather than stockers, to implement testing strategies rather than vaccination. In larger herds and herds that imported stockers, a strategy that included vaccination was always dominant or co-dominant. Herds that import stockers or large numbers of other animals should consider a vaccination strategy. Similarly, herds that have contact with other herds on fencelines or communal pastures have introduction risk that cannot be controlled by testing and should consider a vaccination strategy.

In the 50- and 100-head herds, strategies combining vaccination and/or large amounts of testing (strategies T and Z) cost more than the target value specified. While these strategies may decrease the risk of introducing and spreading BVDV in the herd, they do not appear to be cost-effective in the long term. A more judicious use of targeted testing, with vaccination when necessary, appears preferable from an economic standpoint.

The results presented in Tables 6 and 7, although useful, are limited to a single target value. While this is useful for decision making if the target value is known, different producers may have different levels of acceptable risk, whereupon cumulative distribution functions (CDFs) become useful. In Figure 1(a), it can be seen that the risk of an outbreak exceeding any cost target value between \$10,000 and \$25,000 in that particular herd is lowest if strategy U (vaccinating breeding animals and testing imported adults) is used. Above \$25,000, the risk of exceeding the target is lowest with the addition of testing imported stockers (strategies T and Z) and/or testing and culling calves pre-breeding (strategies Y and Z). The base cost of these programs is greater, but they provide more protection; a risk-adverse producer may choose to use

the more expensive strategy, while another producer may find the increased risk of large costs acceptable in exchange for a less-expensive base cost.

In Figure 1(b), there are primarily two dominant biosecurity strategies evident for herd B3. The less expensive strategy is vaccinating breeding animals and testing imported calves, including the calves of the pregnant imported heifers (strategy V). Additional protection from risk of large outbreaks comes with also testing adult imports and testing and culling calves pre-breeding (strategy Y), but the base cost is higher. For herd C4 (Figure 1(c)), testing only the calves of pregnant imports (strategy P) is dominant at low cost levels, but testing the adult imports and testing and culling the calves pre-breeding, as well (strategy S), is necessary to minimize the risk of outbreaks exceeding \$2,700. Again, the choice of a biosecurity strategy depends on the willingness of the producer to accept either the increased risk of a larger outbreak or the increased cost of a more expensive strategy.

The results presented here are specific to the herd profiles used to obtain them and indicate that the most cost effective biosecurity plan should be designed for the specific risks of the herd. While generalizations may be made, it is preferable to model herds on an individual basis. However, the results presented here suggest that for smaller herds with modest import rates, strategic testing of imports is the most cost-effective way to exclude BVDV and control losses. For herds with larger numbers of breeding animals and/or some level of uncontrolled risk, such as exposure to stockers or neighboring herds, vaccination of the breeding herd may be a cost-effective addition to strategic testing strategies.

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## **Appendix A - Critical Literature Review Checklists**

The checklists used to evaluate literature during the critical review are included below. Categorization of each study was based on the author's opinion. Hybrid studies were analyzed based on the criteria pertinent to each study.

### **Cross-sectional Studies**

- objectives stated adequately
- sample size justified
- groups representative of field conditions
- reason operations declined described
- methods of cooperator recruitment and cooperators described
- animal or pen selection described and justified
- intervention protocols or exposure variable described
- appropriate comparison or control group used
- intervention or exposure assessed equally for cases and controls
- adequate lab tests to determine outcome used
- time from intervention to outcome measurement sufficient
- mortality, withdrawals, lost to follow up reported
- proportion of lost to follow up adequate
- stats analysis appropriate
- estimates and measures of variability used presented adequately
- confounders appropriately considered
- conclusions supported by results

### **Case-Control Studies**

- objectives stated adequately
- sample size justified

- groups representative of field conditions
- reason operations declined described
- methods of selecting case and controls described
- cases and controls similar with equal exposure opportunity
- animal or pen selection described and justified
- intervention protocols or exposure variable described
- appropriate comparison or control group used
- intervention or exposure assessed equally for cases and controls
- adequate lab tests to determine outcome used
- time from intervention to outcome measurement sufficient
- mortality, withdrawals, lost to follow up reported
- proportion of lost to follow up adequate
- stats analysis appropriate
- estimates and measures of variability used presented adequately
- confounders appropriately considered
- conclusions supported by results

### **Cohort Studies**

- objectives stated adequately
- sample size justified
- groups representative of field conditions
- reason operations declined described
- methods of cooperator recruitment and cooperators described
- animal or pen selection described and justified
- intervention protocols or exposure variable described
- appropriate comparison or control group used
- route, schedule, grouping of intervention commercially feasible
- outcome assessor blinded to intervention status of units
- adequate lab tests to determine outcome used
- time from intervention to outcome measurement sufficient

- clinically relevant outcome
- mortality, withdrawals, lost to follow up reported
- proportion of lost to follow up adequate
- stats analysis appropriate
- estimates and measures of variability used presented adequately
- confounders appropriately considered
- conclusions supported by results

### **Randomized Clinical Trials**

- objectives stated adequately
- sample size justified
- groups representative of field conditions
- reason operations declined described
- methods of selecting case and controls described
- methods of cooperator recruitment and cooperators described
- intervention protocols or exposure variable described
- appropriate comparison or control group used
- descriptive stats support valid randomization, numbers per group
- sampling units randomly assigned to the treatment groups
- sampling units tested for outcome prior to intervention
- route, schedule, grouping of intervention commercially feasible
- outcome assessor blinded to intervention status of units
- adequate lab tests to determine outcome used
- time from intervention to outcome measurement sufficient
- clinically relevant outcome
- mortality, withdrawals, lost to follow up reported
- proportion of lost to follow up adequate
- stats analysis appropriate
- estimates and measures of variability used presented adequately
- confounders appropriately considered

- conclusions supported by results

### **Challenge Trials**

- objectives stated adequately
- sample size justified
- intervention protocols or exposure variable described
- appropriate comparison or control group used
- descriptive stats support valid randomization, numbers per group
- sampling units randomly assigned to the treatment groups
- sampling units tested for outcome prior to intervention
- route, schedule, grouping of intervention commercially feasible
- outcome assessor blinded to intervention status of units
- adequate lab tests to determine outcome used
- time from intervention to outcome measurement sufficient
- mortality, withdrawals, lost to follow up reported
- proportion of lost to follow up adequate
- stats analysis appropriate
- estimates and measures of variability used presented adequately
- confounders appropriately considered
- conclusions supported by results

## **Appendix B - Assumptions of the Model**

The model presented in chapters 3-5 makes several assumptions, either for the sake of simplicity or where no data is available.

### **Assumptions for Introduction (Chapter 3)**

- All heifers are assumed to be imported from a single herd, allowing for clustering of PIs.
- Pregnant imports that are PIs are assumed to give birth to PI calves.
- When PI calves are detected among the calves of pregnant imports, it is assumed that their dams will be tested. If any pregnant imports are PIs, they will be included in this testing.
- Animals are assumed to be kept in perfect quarantine before test results are available.
- Test specificity is assumed to be 100%.

### **Assumptions for Spread (Chapter 4)**

- PIs are the only source of infection; horizontal transmission cannot occur from TIs.
- PI mortality is assumed to occur randomly in the period between calving and weaning.
- Calves of resistant dams are assumed to be resistant from birth to weaning.
- Conception rate is assumed to be 65% per 3-week period for herds with a 60-day breeding season and 50% per 3-week period for herds with a 100-day breeding season.
- All births, deaths, infections, and conceptions are assumed to occur on day 1 of the 3-week period in which they occur.
- EED's are assumed to occur according to the distribution in Table 5.1 to dams transiently infected between 1 to 42 days gestation.

- When EED's occur before the last breeding cycle, the dam is assumed to be available for re-breeding.
- PI's are assumed to occur according to the distribution in Table 5.1 to dams transiently infected between 43 to 126 days gestation.
- Fetal malformations are assumed to occur according to the distribution in Table 5.1 to dams transiently infected between 127 days gestation and weaning (252 days after the beginning of calving).
- Abortions are assumed to occur according to the distribution in Table 5.1 to all dams transiently infected on an annual basis.
- PI heifers born in the herd are assumed to be retained at 30% the rate of retention in all heifers.
- Mortality (for both TIs and PIs) is assumed to occur only in morbid animals.
  - Morbidity is assumed to be 100% if the mortality rate is larger than the case fatality rate.
- Resistance from vaccination is assumed to last one year
  - Resistance from transient infection is assumed to last 1 year in 50% of TI animals, and 2 years in the remainder of TIs.
  - Resistance from transient infection is assumed to be total; it is assumed that the strain of BVDV will not be different from year to year.

### **Assumptions for Economic Analysis (Chapter 5)**

- Infection from fence-line contact resulted in a single PI present within the breeding herd for a 3-week period. The time of the infection was modeled randomly within the period between calving and weaning.
  - It is assumed that the  $R_0$  will be lower (Table 5.1) if the infection is due only to fence-line contact, but the  $R_0$  will be chosen from the higher distribution if infection is present in the herd from any other source.
- Imported PI fetuses are randomly assigned to birth in 3-week periods during the calving season.
- All other PI imports are assumed to be added to the herd on the first day of the calving season.



- Treatment cost is applied to all morbidities.
- Labor costs are based on hourly costs.
  - Vaccination is assumed to require 0.033 hours (2 minutes) per animal in herds of  $\leq 50$  head, 0.017 hours (1 minute) per animal in herds of 51-100 head, and 0.008 hours (1/2 minute) per animal in herds of  $>100$  head.
  - Testing is assumed to require 0.017 hours (1 minute) per animal in all herds.
- Weaning weights for steers and heifers are strongly correlated in each year.
- The price draws for steers and heifers are strongly correlated within each year, as are the price draws between weaning weights.
- In the results presented here, it is assumed that calves are marketed at weaning and that weaning occurs in September.

## Appendix C - Expert Survey Questionnaire

The following questionnaire was sent to 5 expert DVMs in the field of BVDV. Their responses were averaged to provide the parameters for pert distributions. Input distributions that were referenced to expert opinion and are not included in this list were defined in a workshop with 4 expert DVMs in cow-calf herd health management.

Dear Dr \_\_\_\_

As part of a Master's degree here at Kansas State University I am building a risk analysis model for BVD biosecurity on cow-calf farms in the US, incorporating both the risk of importing BVD and the impact it will have on individual herds, based on their management practices. In the course of the literature review, a number of holes in our understanding of BVDV transmission were noted.

Please take a moment to answer any or all of these questions below based on your experience with BVDV. For each question, it would be useful to have estimates for the minimum, maximum, and most likely values.

Please fill in the blanks below and return to the email address below.

Thank you very much for your time and expertise

Becky Smith DVM (rebecca.lee.smith@gmail.com),

Mike Sanderson DVM, MS DACVPM (Epidemiology)

1. What is the PI mortality rate:
  - a. from birth to 60 days (What proportion of PI calves die between birth and 60 days of age?)
    - i. minimum:
    - ii. maximum:
    - iii. most likely:

- b. from 60 days to weaning (What proportion of PI calves die between 60 days of age and weaning?)
    - i. minimum:
    - ii. maximum:
    - iii. most likely:
  - c. from weaning to 45 days post-weaning (What proportion of PI calves die between weaning and 45 days post weaning?)
    - i. minimum:
    - ii. maximum:
    - iii. most likely:
2. What is the percent increase in morbidity rate in non-PI calves when PIs are present in a herd?
- i. minimum:
  - ii. maximum:
  - iii. most likely:
3. What is the percent increase in mortality rate in non-PI calves when PIs are present in a herd?
- i. minimum:
  - ii. maximum:
  - iii. most likely:
4. Among dams infected before 45 days gestation, what is the proportion of early embryonic deaths?
- i. minimum:
  - ii. maximum:
  - iii. most likely:
5. Among dams infected after 125 days gestation, what is the congenital defect rate in calves?
- i. minimum:
  - ii. maximum:
  - iii. most likely:

6. What is the probability of infection from fence-line contact with neighboring infected herds?
  - i. minimum:
  - ii. maximum:
  - iii. most likely:
  
7. How many susceptible animals will be infected by a single PI in the herd over a 21-day period?
  - i. minimum:
  - ii. maximum:
  - iii. most likely:
  - b. Is it different for PIs with fence-line contact? If so, what is the number infected?
    - i. minimum:
    - ii. maximum:
    - iii. most likely:
  
8. What is the proportion of susceptible dams that will abort in a year from BVD infection if BVD is present in the herd?
  - i. minimum:
  - ii. maximum:
  - iii. most likely: