THE PERSISTENTLY INFECTED BOVINE VIRAL DIARRHEA VIRUS INDIVIDUAL:
PREVALENCE, VIRAL SURVIVAL, AND IMPACT WITHIN COMMERCIAL FEEDING
SYSTEMS

by

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B.S., South Dakota State University, 1999
M.S., South Dakota State University, 2002
D.V.M., Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

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Abstract

Bovine viral diarrhea virus (BVDV) has emerged as one of the most important infectious diseases in cattle. One particular important manifestation, after successfully establishing an in utero infection of the fetus during the first trimester, is the development of a persistently-infected BVDV (PI-BVDV) calf. Persistently infected BVDV animals are a continuous source of virus and can shed the virus in virtually all secretions and excretions, including nasal discharges, saliva, semen, urine, tears, milk, and, to a lesser extent, feces. The objectives of this research were to determine: 1) the effects of short term exposure (13 – 18 days on feed (DOF)) to PI-BVDV feeder cattle; 2) the outcome of testing and removing PI-BVDV feeder calves at time of feedlot arrival on health, performance, and carcass characteristics; 3) the survival of BVDV on materials associated with livestock production; and 4) characterization of testing and longitudinal prevalences for PI-BVDV beef cattle. Testing and removing PI-BVDV calves at 13 to 18 DOF was too late to remove a morbidity effect due to PI-BVDV exposure. However, mortality, performance, and carcass characteristics were not different in cattle exposed to PI-BVDV cattle. Additionally, there were no harmful outcomes when newly arrived feeder cattle were exposed to a PI-BVDV animal for one to two days following feedlot entry. A non-cytopathic, Type 1b, BVDV was capable of surviving after application to various materials used in livestock production. BVDV tended to survive longer on non-porous materials than porous materials. When in the presence of mucus, BVDV was protected from degradation for longer periods of time than when not in the presence of mucus. There was no difference in overall PI-BVDV prevalence within cattle sampled in 2006 and 2007. Cattle that weighed less than 300 lbs. had a greater likelihood of being PI-positive than cattle with increased weights. Several months of the year had a greater likelihood of having PI-positive animals. Based on operation, cow-calf and

stocker operations had a greater likelihood of having PI-positive animals than did feedlot

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CHAPTER 1 – LITERATURE REVIEW

Pestivirus

Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus* within the family Flaviviridae, which also contains the genera *Flavivirus* and *Hepacivirus* (Brock et al., 1992). Other members of the genus are the important animal pathogens *Classical swine fever virus* and *Border disease virus* of sheep. Pestiviruses are enveloped, single stranded, positive-sense RNA viruses with genomes of ~12.3 kb that contain one long open reading frame (ORF) coding for a polyprotein of about 4,000 amino acids, which is co- and post-translationally processed into at least 12 mature proteins (Lackner et al, 2004; Lindenbach et al, 2001). The proteins C, E^{rns}, E1, and E2 are structural components of the virion. Additionally, the E^{rns} and E2 proteins induce neutralizing antibodies in infected animals and elicit protective immunity (Rumenapf et al, 1993).

Cytopathic (cp) and noncytopathic (ncp) biotypes of all pestivirus species can be differentiated during replication in tissue culture systems. The cp phenotype is characterized by a loss of control of genome replication and reduced ability of the infected cell to prevent a type 1 interferon (IFN) response to double stranded RNA (dsRNA). The genome of the pestitviruses are similar to human hepatitis C virus, in that they both have a similar gene expression, biochemical properties and similar functions for the viral proteins. A few differences do exist, one of which is the coding of two additional proteins by the pestivirus RNA. The two extra proteins are the N^{pro} and E^{rns}. The N^{pro} is a protease which interferes with the host interferon (IFN) response, which is critical for anti-viral effectiveness. The E^{rns} has a dual purpose function; it is an important component of the viral envelope and it has intrinsic RNase activity. The E^{rns} is also secreted into the extracellular spaces. Deletion of the N^{pro} causes attenuation,

whereas deletion of the E^{rns} allows viral replication to occur, but there is no infectious virus produced.

Bovine Viral Diarrhea Virus

In 1946, bovine viral diarrhea (BVD) was first reported, but it took another several years to identify the responsible virus, which was designated bovine viral diarrhea virus (BVDV) (Olafson et al, 1946; Baker et al, 1954; Pritchard et al, 1955; Underdahl et al. 1957). There are two genotypes of BVDV, type 1 and type 2, and their nucleotide sequence homology is roughly 60% similar. Within type 1 there are eleven subgenotypes; however, type 1a and 1b are the most prevalent. Within type 2 there are two subgenotypes, type 2a and 2b (Ridpath et al. 1994). In addition to classification by subgenotype, BVDV is also classified by biotype. The biotype classifications for BVDV are noncytopathic (ncp) and cytopathic (cp). The characterization is based on the presence or absence of cytopathic effect in cell culture systems (Qu et al, 2001). Noncytopathic biotypes are more prevalent in nature and are believed to be the origin of cytopathic strains.

Clinical Symptoms of BVDV

The clinical manifestations of BVDV are diverse and vary in severity, duration, and the organ system involved. In cattle seronegative and immunocompetent to BVDV, the majority of BVDV infections are subclinical. It has been estimated that 70 to 90 % of BVDV infections occur without clinical manifestations (Ames, 1986). After infection, the upper respiratory tract and lymphoid tissue are sites for viral replication (Bolin, 1990). Cattle undergoing subclinical infection may demonstrate a mild elevation in body temperature and leukopenia. Subclinical BVDV infections are likely the reason for the high prevalence of seropositive cattle (Harkness et al, 1978).

As the infection becomes clinical, the name transitions to bovine viral diarrhea (BVD). Typically animals are 6 months to 1 year of age when clinical disease presents, which is characterized by high morbidity and low mortality. The incubation period is 5 to 7 days and is followed by transient fever and leukopenia. Viremia occurs 4 to 5 days after infection and can persist for up to 15 days (Brownlie et al, 1987; Duffell et al, 1985). Clinical findings include depression, anorexia, oculonasal discharge, occasionally oral lesions characterized by erosions and ulcerations, diarrhea, and a decrease in milk production.

Given that BVDV is an RNA virus, the genomic diversity creates some strains that are more pathogenic to the host than others. Infection with a type 2 BVDV is usually more severe than a type 1 BVDV. Many of the clinical signs are similar for type 1 and type 2 BVDV; however, one difference for type 2 BVDV is the development of thrombocytopenia during an infection (Nagele, 1984). Furthermore, noncytopathic BVDV strains are more commonly associated with thrombocytopenia. The clinical consequence of thrombocytopenia is bloody diarrhea, epistaxis, petechial and ecchymotic hemorrhages on mucous membranes, and bleeding from injection site (Rebhun et al, 1989).

In the early 1990's there were several reports of an increase in the severity of acute clinical cases of BVDV. These clinical cases where associated with an acute onset of diarrhea, pyrexia, decreased milk production, morbidity near 40 % and mortality ranged between 10 to 25 % (David et al, 1994; Hibberd et al, 1993; Carmen et al, 1994). Many of the viral isolates were noncytopathic and were typed as a type 2 BVDV. Interestingly, during the time frame that these severe acute BVDV cases where occurring, commercial BVDV vaccines only contained a type 1 BVDV. Serologic data has suggested that there is relatively low cross reactivity between type 1 and type 2 BVDV (Pellerin et al, 1994). In light of this, there were concerns about the sufficient

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cross protection from type 1 BVDV vaccines. However, a BVDV type 1 modified live vaccine given to 2-week old calves protected them from a later challenge with a type 2 BVDV (Ellis et al, 2001).

BVDV Immunosuppression

It has been well established that acute BVDV infections can result in immunosuppression of the host (Potgieter, 1988). The important aspects of BVDV induced immunosuppression is that the host's susceptibility to other pathogens is increased and the pathogenicity of co-infecting pathogens may also be enhanced. The ability of BVDV to cause immunosuppression contributes to a broad range of clinical diseases (Baker, 1995).

BVDV and Bovine Respiratory Disease

The majority of evidence is suggestive of a role for BVDV in bovine respiratory disease (BRD). In one study, BVDV was the virus most often demonstrated from pneumonic lungs of feedlot cattle and usually was found in association with *Mannheimia haemolytica* (Reggiardo, 1979). Additionally, an association between BVDV antibody titers and treatment for BRD has been reported (Martin et al, 1986).

BVDV Infections in Immunocompetent, Pregnant Cattle

In addition to the typical clinical manifestations of BVD described earlier, transplacental transmission to the conceptus is possible in cattle (Duffell et al, 1985). The principle determinant of the outcome of fetal infection is the gestational age at the time transplacental infection occurs. Although one outcome of BVDV infection of pregnant cattle is the development of an immunotolerant calf, infection of pregnant cattle also has other outcomes. If infected at the time of conception, there are reduced conception rates. Also, within the first 4 months of gestation, there is the potential for embryonic resorption, abortion, or growth

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retardation. Infections that occur at 4 to 6 months of gestations are associated with central nervous system disorders and congenital malformations. Fetal mummification, premature birth, stillbirth, and weak calves can also occur. Both cytopathic and noncytopathic biotypes can cause fetal infections and fetal loss; however, the noncytopathic biotype is more common (Dubovi et al, 1992). Additionally, it is the noncytopathic biotype that produces immunotolerant persistently infected BVDV animals (McClurkin et al, 1984).

Mucosal disease is a highly fatal form of BVD, which may be acute or chronic and is seen most frequently in persistently infected cattle. The disease develops when a cytopathic BVDV, which is often an escape mutant from the animal's own noncytopathic BVDV from the fetal infection, is antigenically similar to the noncytopathic internal BVDV. Since the individual is immunotolerant to the ncp BVDV, the individual's immune system does not recognize the external cp BVDV. It is also possible for mucosal disease to occur with an external cp BVDV (i.e. non-mutated ncp BVDVs); this can be discriminated by serology as these animals will have a serum titer to BVDV.

Persistent Infection

In the field, pestiviruses establish persistent infections in their natural hosts. BVDV is a successful pathogen that can cause a persistent infection. Approximately 0.5 to 2 % of newborn calves in dairy herds are persistently infected (Houe, 2003). The early period of gestation is essential because if the fetus is infected later in the gestational period the fetus can use the adaptive immune system to eliminate the BVDV infection.

Fetuses that survive infection with noncytopathic BVDV between 18 and 125 days of gestation are at significant risk for developing immunotolerance to the virus and subsequently become persistently infected (PI) with BVDV. The first published account of a PI-BVDV

animal was in 1978 (Coria et al, 1978). In that paper, Coria et al. described the isolation of BVDV from an animal that was seronegative to BVDV. In light of these findings, they sought to answer how and under what circumstances this infection could have taken place. They completed five experiments using five different BVDV isolates: 1) Two –BVDV isolates (7443 (ncp) & NY-1 (ncp)) were inoculated into seventeen fetuses of BVDV seropositive cows at 58 to 125 days of gestation; and 2) Three BVDV isolates (VM (ncp), MC (ncp –cp), and NADL (cp)) were inoculated intravenously into 44 BVDV seronegative cows at 42 to 144 days of gestation. The results indicated that the birth of calves PI with BVDV can result from exposure of a BVDV negative dam to BVDV between 42 and 125 days of gestation. Interestingly, the use of the cp BVDV NADL-isolate did not cause a calf to be PI with BVDV; however, the use of a ncp BVDV isolate did cause calves to be PI with BVDV (McClurkin et al, 1984). This was the first formal recognition that ncp isolates were able to induce immunotolerance during a specific gestational timeline.

Infection of cells with viruses induces the production of interferons. The inteferons associated with anti-viral effects are interferon-α (IFN-α), and interferon-β (IFN-β). Interferons are induced by double stranded RNA (dsRNA), which is not found in mammalian cells, but is an important part of the genomes of some viruses. Interferon-α and IFN-β have three major functions: 1) They induce resistance to viral replication by upregulating expression of proteins that destroy messenger RNA (mRNA) and inhibit downstream translation of viral proteins; 2) They induce the expression of major histocompatibility complex class 1 (MHC class 1) and make virally infected cells more recognizable to cytotoxic killing by CD8+ T-cells; and 3) They activate natural killer (NK) cells, which then kill virally infected cells.

The exact mechanism(s) by which the noncytopathic isolates induce persistent infection is not clear. To persist in an individual, BVDV must overcome the host's immune response. The type 1 interferons (IFN), commonly referred to as alpha/beta interferons (IFN α/β), are an important cellular mechanism that are stimulated in response to viral infections. If produced, they place the cell in an antiviral state. It not only does this for its own cell, but it is secreted and can protect other cells against viral infection. The downstream signal transduction following upregulation of IFN-α/β is prolific (Der et al., 1998). The better characterized antiviral proteins induced by IFN-α/β are protein kinase PKR and 2', 5'-oligoadenylate synthetase, which are both activated by dsRNA and Mx protein. Since the induction of IFN-α/β is detrimental to viral propagation and survival, many viruses have developed mechanisms to avoid the establishment of the antiviral state afforded by upregulation of IFN-α/β. For example: 1) Some viruses prevent the induction of IFN synthesis; 2) Some viruses prevent the action of IFN after production; 3) Some interfere with signal transduction pathways activated after IFN binds its cellular receptor; and 4) Many viruses inhibit the activity of IFN-induced antiviral proteins (Schweizer et al, 2006).

Several laboratories have attempted to better understand the interplay between cp and ncp BVDV isolates with the immune system. Cells infected with ncp BVDV do not undergo apoptosis, which is commonly referred to as programmed cell death (Adler et al, 1997; Perler et al, 2000; Schweizer et al, 1999; Zhang et al, 1996). However, if a cell is infected first with a ncp BVDV and later infected with another virus, the cell will undergo apoptosis (Baigent SJ et al, 2002; Schweizer et al, 2001). Through the use of extensive in vitro systems, it has been shown that ncp BVDV inhibits the production of IFN (Adler et al, 1997; Perler et al, 2000; Schweizer et al, 2001). This important *in vitro* finding has been confirmed using an *in vivo* BVDV model

(Charleston et al., 2001). In that study, fetuses that were challenged with a non-cytopathic isolate failed to mount an IFN response and subsequently developed a persistent infection. While being resistant to induction of IFN, cells infected with ncp BVDV are capable of responding to exogenous IFN with a robust antiviral state that protects from infection with unrelated viruses without eliminating the ncp BVDV residing in the same cell (Schweizer et al, 2001). There have been several experiments completed to better understand the defects of the immune system with regards to recognition and clearance of homologous BVDV in PI-BVDV calves. Interestingly, antigen presenting cells (APC) from a PI animal could stimulate a T-cell response to homologous virus. To the researchers, this demonstrated that the mechanism associated with persistence did not involve antigen presentation (Glew et al, 2001).

Laboratory Diagnosis

There are several testing methodologies available to assist in diagnosing BVDV; however, not all methodologies are applicable to all clinical situations. For instance, the testing of acute infections manifested with clinical disease, reproductive failure, detection and elimination of PI individuals, testing for vaccine efficacy, biological products which require quality control and genotyping are situations where the testing methodology is important (Saliki et al, 2004).

There are a number of diagnostic tests available to the practitioner. The gold standard is virus isolation. A number of clinical specimens can be submitted for virus isolation. Frequently, the clinical specimen will be a tissue or a serum specimen. The clinical specimen with greatest opportunity for diagnosis is the white blood cell fraction from a whole blood sample.   When tissues must be used for virus isolation, the lymphoid organs, such as spleen, Peyer's patch, mesenteric lymph nodes, and thymus are the most appropriate.   The use of serum can be

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associated with variable results due to the potential presence of neutralizing antibodies. After processing, an aliquot of the specimen is transferred into a cell culture system. The most commonly used cell lines for virus isolation of BVDV are Mandin Darby Bovine Kidney (MDBK) cells, bovine turbinate (BT) cells, and bovine testicle (Btest) cells. After a range of incubation times, typically 3 – 5 days, the cells are visualized to determine if they are infected with BVDV. Cytopathic strains are easy to identify due to the destruction of the monolayer of cells. On the other hand, a non-cytopathic strain will have a normal monolayer. Therefore, it is necessary to use a monoclonal antibody with specificity for identification and confirmation of BVDV.

A cheaper and faster detection time can be accomplished by detection of BVDV antigen. One concern with antigen detection is a lack of sensitivity and reliability when compared to virus isolation. Therefore, antigen detection methods are most often used for screening animals. Currently, there are three testing options: antigen capture enzyme linked immunosorbent assay (ACE); immunologic staining of fresh or formalin fixed tissues; and PCR. As these tests are used as screening tools, they are employed for the identification of PI animals. One limitation is that the tests may not differentiate an animal with an acute infection from a PI animal. Immunologic staining includes tissues samples, with probably the majority of the tests performed on ear notch biopsies.

With the advent of polymerase chain reactions (PCR), a number of valid PCR tests have been developed by a variety of testing centers. Given that BVDV is an RNA virus, the PCR technology most commonly employed is reverse transcriptase PCR (RT-PCR). Most samples can be used for RNA isolation; however, formalin fixed tissues can be an issue. In one study, when BVDV infected tissues where held in 10 % formalin for 10 days, there was a loss of

detection.   If 5 % formalin was used, it extended the shelf-life to 3 months. One limitation of

PCR is a high cost. To reduce the costs of PCR testing, pooling of samples is often allowed.

Pooling is often used for serum samples and for bulk tank testing to identify the presence of a PI

animal. Another advancement in PCR technology is the development of real time PCR (rtPCR).

Real time PCR takes advantage of molecular beacons that have a fluorescence tag which emits

when they are cleaved by Taq polymerase. In addition to the two standard 5' and 3' primers, an

additional primer is developed that is labeled with a flurochrome. The additional primer

corresponds to an area between the 5' and 3' primers. If the target is present in the sample, all

the primers will bind to the DNA. As the Taq polymerase adds the nucleotides to create the

complementary DNA, it cleaves the flurochrome present on the additional primer and the

flurochrome is released into the rtPCR solution. This flurochrome is then measured with a

spectrophotometer and graphed. This technology allows for not only identification, but also for

quantification of the number of viral transcripts.

Serology is used for the measurement of antibody response in animals with natural

exposure or after vaccination. Two common testing methods are ELISA and serum

neutralization. ELISA is the less preferred of the two tests largely due to viral diversity. On the

other hand, the use of a serum neutralization assay is not without pitfalls. Serum neutralization,

which provides a titer of antibody, is subject to variation by the strain of BVDV that is used and

the test cells. Lastly, because of a lack of standardization across diagnostics laboratories, not all

diagnostic laboratories use the same BVDV and/or cells. Therefore, there is the chance that

there can be a discrepancy between testing laboratories. The strengths of the serum

neutralization assay, when applied appropriately, are to: 1) assess vaccine efficacy; 2) assess

vaccination protocol compliance; 3) assess herd status as to exposure to BVDV; and 4) associate BVDV with clinical signs.

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CHAPTER 2 - Effects of Short Term Exposure of Feeder Cattle to Calves Persistently Infected with Bovine Viral Diarrhea Virus

ABSTRACT

A research trial involving auction derived feeder steers and heifers (n = 2,954) was conducted at a single commercial feedlot in Kansas to determine the effects of testing cattle persistently infected (PI) with bovine viral diarrhea virus (BVDV) at revaccination (10 – 14 days on feed (DOF)) and removing them (13 – 18 DOF) from the home pen. After removal of test positive animals, the effect of the short term exposure (STE) on the health, performance and carcass characteristics was determined. The percentage of calves exhibiting signs of morbidity was increased in cattle with STE to PI-BVDV (P < .01). Cattle that had no exposure (NE) to PI-BVDV calves had a morbidity of 19 % compared to a 30 % morbidity rate observed in pens with STE to a PI-BVDV calves during the feeding period (P < .01) Further characterization of the temporal pen morbidity of STE and NE revealed that 31.7 % of all STE and 15.3 % of NE pulls occurred during 0 - 7 DOF. Additionally, the incidence of cattle treated for Bovine Respiratory Disease (BRD) in STE calves was 2.17 initial treatments per 1,000 head-days at risk (95% CI 1.73 to 2.72) whereas the incidence of treatment in NE was 1.28 initial treatments per 1,000 head-days at risk (95% CI 0.98 to 1.68). Short term exposure to PI-BVDV had no effect on the retreatment rate (P = .82), death loss (P = .69), or performance (P > .05). There was no evidence of a BVD PI exposure x sex interaction (P = .62) for carcasses that graded choice or better, but there was a main effect of sex (P < .01). There was evidence of a BVD-PI exposure x sex interaction for Yield grade 2 or greater carcasses (P=.03). Testing at 10 - 14 DOF was too late in

the feeding period to eliminate the observed initial morbidity spike that occurred during 0 – 7 DOF which resulted in the overall morbidity differences between calves with STE or NE.

INTRODUCTION

Infection with bovine viral diarrhea virus (BVDV) contributes to a variety of economically important disease syndromes in beef cattle, including bovine respiratory tract disease and immunosuppression of stocker and feedlot cattle [3]. The outcome of BVDV fetal infections in susceptible heifers and cows is dependent on the age of the fetus when exposed. Infection can result in abortion, stillbirths, congenital malformations and birth of persistently infected (PI) calves. Persistent infection in a calf develops when a susceptible heifer or cow is exposed to a noncytopathic BVDV during pregnancy at approximately 45 to 125 days of gestation[5]. Cattle persistently infected with BVDV can shed copious amounts of BVDV into the environment through secretions and excretions, including nasal discharges, saliva, semen, urine, tears, and to a lesser extent, feces.

Relatively few cattle are PI on arrival at a feedlot. The prevalence of PI-BVDV feeder cattle entering feedlots is estimated to be 0.3 % [4]. PI cattle are important sources of virus for animals in direct or close contact. In a recent study, the risk of initial treatment for bovine respiratory disease (BRD) was 43 % greater in cattle exposed to a PI calf [5]. Persistently infected calves tend to have lower growth rates and often die from classic mucosal disease or other diseases during the feeding period [10].

Given the increased risk of morbidity associated with exposure to PI calves, it is important to understand the impact of identifying and removing these animals to limit their exposure to cattle and limit costs associated with treatment (labor and medicine). Therefore, our

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objective was to identify (10 – 14 days on feed (DOF)) and remove (13 – 18 DOF) positive PI-BVDV feeder cattle to determine what effect short term exposure to PI-BVDV has on health, performance, and carcass characteristics of cattle within a pen that contained a PI animal.

MATERIALS AND METHODS

Cattle and sample collection

Auction derived steers (n = 932) and heifers (n = 2,022) (total head = 2,954) arrived at a 12,000 head capacity feedlot in Kansas between 8/24/05 – 11/15/05. Upon arrival, the cattle were administered doramectin[a], a modified live vaccine containing bovine herpesvirus type 1, parainfluenza type 3, BVDV (Types 1 and 2) and bovine respiratory syncytial virus[b]. In addition, a steroid implant[c] was administered subcutaneously in the caudal aspect of the ear. After initial processing, cattle were housed in 19 pens (range 52 - 255 head/pen) and managed in accordance with routine feedlot practices.   Ten to fourteen days after processing, the cattle received a second MLV vaccination[d] and a multivalent clostridial vaccine[e]. At revaccination, fresh skin (ear notch) specimens were collected and placed in phosphate buffered saline solution and tested for BVDV antigen by antigen capture ELISA (ACE). During the feeding period, animals exhibiting symptoms consistent with respiratory disease were removed for diagnosis and treated with antimicrobials if necessary. The first, second, and third antimicrobial treatments for BRD were tulathromycin[f] (Draxxin, Pfizer Animal Health), florfenicol[g] (Nuflor, Schering Plough Animal Health), and ceftiofur hydrochloride[h] (Excenel, Pfizer Animal Health), respectively.

Antigen Capture ELISA

Detection of BVDV antigen in skin specimens (ear notch) was performed by use of a commercial antigen capture ELISA (ACE) kit[i]. Results were calculated by the following equation: standardized OD = (raw OD of sample – raw OD of negative control)/(raw OD of positive control – raw OD of negative control). Samples with standardized OD values < 0.20 were considered negative, and those with OD values > 0.39 were considered positive. Samples with values from 0.2 to 0.39 were retested with detector reagents with or without antibody. If an animal tested positive at revaccination, it was removed from the pen, isolated, and retested 21 days later by immunohistochemistry (IHC) on an ear notch biopsy for BVDV for confirmation of PI status.

Treatment Groups

If an animal tested positive for BVDV by ACE, it was removed from the home pen (range = 13 – 18 DOF). Twenty one days after the original ACE test, confirmation of ACE positive animals was performed with IHC. After confirmation of BVDV PI positive status, the home pen of origin was considered to have had short-term exposure (STE) to PI-BVDV animals. Pens with STE were compared to pens that had no exposure (NE) to PI-BVDV. Short term exposure and NE pens were followed through closeout and slaughter.

Health and Performance Data

Feedlot data were collected from electronic records maintained at the feedlot.   Data obtained from the close-out sheets included initial weight, final weight, days on feed, average daily gain, dry matter intake, feed to gain, and feed cost of gain per pound.   Health data were

recorded daily by trained feedlot personnel. Data gathered from the animal health computer[j] included respiratory morbidity, number of treatments, death loss and treatment costs.

Statistical Analysis

Data were analyzed using commercially available statistical analysis software[k]. Pen-level response variables were generated and analyzed using regression techniques. Continuous response variables were analyzed using general linear models whereas discrete binomial response variables (events/trials) were analyzed using generalized linear models with a logit link function. The interaction of exposure and sex was evaluated, and, if not significant, removed from the model. Further, if the main effect of sex was not significant, it too was removed from the model while the main effect of exposure was forced into the model. A time by exposure effect on counts of initial treatment was evaluated using negative binomial models and repeated measures methodologies in that within-pen dependency over time was modeled using compound-symmetry matrices. Model predicted estimates of incidence of initial treatment for respiratory disease and associated confidence intervals were calculated from the final model.

RESULTS

Of the 2,954 head that were tested for PI-BVDV, 10 calves were positive (prevalence = 0.35%). At the pen level, 5 of the 19 pens had exposure to a PI-BVDV calf (26.3%). The percentage of calves exhibiting signs of morbidity was increased in animals with short term exposure (STE) to PI-BVDV (P = .01; Table 2.1). The temporal pattern of morbidity was investigated. Cattle in the STE pens had increased morbidity relative to the cattle in the NE pens during the first seven days on feed (Figure 2.1). There was no evidence of a BVD PI exposure x

period interaction (P = 0.25), but there was a main effect of BVD PI exposure on morbidity rates (P=0.02). The incidence of BRD treatments in STE cattle was 2.17 initial treatments per 1,000 head-days at risk (95% CI 1.73 to 2.72) whereas the incidence of BRD treatments in NE cattle was 1.28 initial treatments per 1,000 head-days at risk [95% CI 0.98 to 1.68 (Figure 2.2)]. Short term exposure to PI-BVDV calves had no effect on the percentage of calves that were retreated (P= .82). Additionally, STE cattle had similar death loss relative to NE cattle (P= .63).

A BVD PI exposure x sex interaction was observed for the final weight of finished cattle. Upon further examination, heifers with no exposure to a PI-BVDV animal had an increased final weight compared to heifers with STE to a PI-BVDV animal. Steers with STE to a PI-BVDV had heavier final weights than steers with no exposure. This is attributed to the finding that steers with STE were fed on average 22 days longer than animals with no exposure. Therefore, the main effects of STE or NE to a PI BVDV animal are presented.

Average daily gain was similar in STE calves relative to NE calves (P= 0.34; Table 2.2). Also, during the feeding period, daily dry matter intake (DMI) was similar in the STE calves relative to cattle never exposed to a PI-BVDV calf (P= .33). There was no difference in the feed to gain ratio (F:G) (P= .57) between the two BVDV exposure groups. No difference was found in the cost of gain for calves regardless of BVDV exposure was observed (P= .24). There was no evidence of a BVD PI exposure*sex interaction (P = .62) for carcasses that graded choice or better, but there was a main effect of sex (P < 0.0001). There was evidence of a BVD PI exposure*sex interaction for yield grade 2 or greater carcasses (P=.03).

DISCUSSION

The current study was conducted to measure the effects of testing and removal of PI-BVDV feeder cattle after arrival in the feedyard. Based on the morbidity results, testing at 10 - 14 days on feed was too late in the feeding period to eliminate the observed initial morbidity spike that occurred during 0 – 7 DOF. Short term exposure to PI-BVDV had no effect on retreatment rate, death loss, performance or carcass characteristics.

Nineteen percent of the NE cattle and 30 % of STE cattle exhibited morbidity during the feeding period. This is different than other published reports [4, 7]. Loneragan et al. observed that there was no difference in morbidity rate for cattle within a pen that contained a PI animal and those pens that did not contain a PI animal [4]. In a second analysis by Loneragan et al., morbidity was defined more broadly to include those cattle considered exposed in the first analysis and those cattle in pens adjacent to a pen containing a PI animal. From their second analysis, cattle exposed to a PI animal had a 43% greater risk of treatment for respiratory disease [4].

In the study reported here, short term exposure to a PI-BVDV had no effect on performance. This was an unexpected finding given the increased morbidity that was observed during 0 – 7 DOF, this has been repeatedly established as having an effect on animal performance. A summary of results from the Texas A&M Ranch to Rail program (1992 – 1997) revealed that animals treated for health complications during the finishing period had 0.05 – 0.25 kg lower ADG than their counterparts that were not treated [6]. Similar reductions in ADG in cattle treated for respiratory diseases has been reported elsewhere [Van Donkersgood et al. 1992 (1.11 vs. 1.25 kg/d)] [11]. Additionally, Hutcheson et al. and Sowell et al. have reported that morbid cattle require increased time after arrival to begin consuming feed when compared to their healthy counterparts [2, 9].

A feedlot finishing study by Gardner et al. reported that steers treated for respiratory disease had lower final live weights, ADG, hot carcass weights, less external and internal fat, and more desirable yield grades. Additionally, steers that were treated had a higher prevalence of carcasses that graded U.S. Standard than steers that were never treated[1]. Similar results were reported in a feedlot-arrival modified live viral vaccine study in which morbidity was improved but there was no improvement in carcass characteristics[8]. The results from the study indicated that there were no significant differences between the experimental groups in any of the carcass characteristic variables that were measured despite noting significant differences in morbidity between the treatments[8].

Clinical signs of BRDC and transient viral infections can present similarly in feeder cattle. There is no mechanism in feedlot production settings for clinical differentiation of fever of unknown origin in feeder cattle. Both the current study and the study by Schunicht were primarily looking at viral exposure and viral vaccination protocols. It is conceivable to the authors of this paper, that transient viral infections might be less detrimental to the overall health and performance of cattle relative to exposure to bacterial pneumonia diseases. Further classification of BRDC etiology and its effects on performance are justified.

The total morbidity between the STE and NE groups was different. Based on these data, 11 % more cattle would be treated with antibiotics in 100 head pens containing a BVDV PI calf verses non-PI pen with similar head count. In our study, tulathromycin was used as the first treatment for respiratory disease. Considering the cost of tulathromycin ($3.45/cwt), the weight of the morbid calves (600 lbs.) and the extra number of head needing treatment (n=11), the cost for medicine alone is $228 greater than in the NE group. If testing earlier removed this morbidity effect, there would have been a savings of $228/100 head or $2.28/head in medicine

costs alone. The cost of testing to identify PI-BVDV animals may potentially be returned or offset by reducing the medicine costs alone. Conceivably, testing and removing PI cattle prior to, or 1 to 2 days after arrival, may decrease the morbidity difference in pens exposed to PI animals to the level of non-PI pens.

Testing and removing PI-BVDV calves at 13 to 18 days on feed was too late in the feeding period to remove a morbidity effect due to PI-BVDV exposure. However, death loss, performance and carcass characteristics were not different in cattle exposed to PI-BVDV cattle compared to cattle never exposed. Therefore, further characterization of the optimal time for testing and removal of PI-BVDV feeder cattle is needed to minimize morbidity for in-contact cattle.

Endnote

[a]Dectomax injectable, Pfizer Animal Health, New York, NY

[b]Arsenal 4.1, Novartis Animal Health, Greensboro, NC

[c]Component E-S, Vetlife, West Des Moines, IA

[d]Vista 5 SQ, Intervet, Millsboro, DE

[e]Vision 7, Intervet, Millsoboro, DE

[f]Draxxin, Pfizer Animal Health, New York, NY

[g]Nuflor, Schering Plough Animal Health, Union, NJ

[h]Excenel, Pfizer Animal Health, New York, NY

[i]Idexx Laboratories Inc., Westbrook, ME

[j]Walco International, Amarillo, TX

[k]SAS System for Windows 9.1.3, SAS Inst. Inc., Cary, NC

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Table 2.1. Effect of short term exposure to persistently infected BVDV cattle on feeder calf morbidity and mortality.

Item	NE	STE	SEM	P-value
Morbidity (%)	18.8	29.6	5.4	<.01
Retreat (%)	14.4	21.7	4.7	.82
Mortality (%)	4.3	5.6	1.8	.69

Table 2.2. Effect of short term exposure to persistently infected BVDV cattle on feeder calf performance.

Item	NE	STE	SEM	P-value
In wt., (lbs.)	626	599	30.2	.44
Out wt., (lbs.)	1142	1163	16.2	.28
DOF, (d)	197	219	11.4	.10
ADG, (lb./d)	2.53	2.46	0.07	.34
DMI, (lb./d)	16.4	15.7	0.68	.33
FG, (lbs. feed/lb. of gain)	6.5	6.4	0.17	.57
COG, ($/lb.)	60.	.62	0.01	.24

Figure 2.1. Temporal morbidity of feeder cattle with or without exposure to PI-BVDV. The percent of total respiratory morbidity (y-axis) that occurred during the feeding period was plotted against days on feed (x-axis).
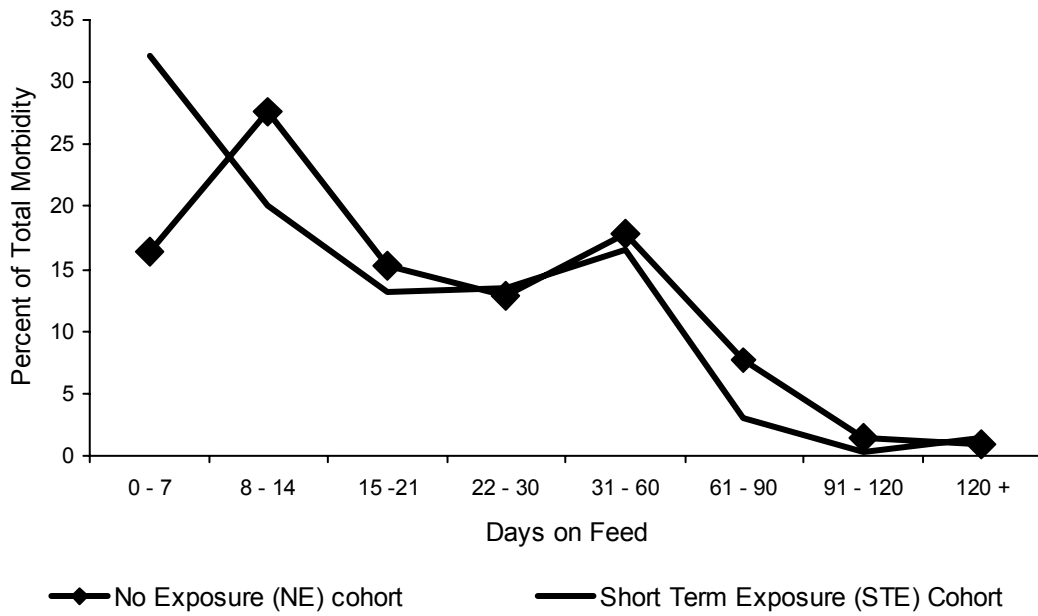
Figure 2.2. Epidemic curve for cohorts either exposed or not exposed to an animal PI with BVDV among feedlot cattle. Exposure was defined to include cattle in a pen that contained a PI animal. Data for the epidemic curve are number of initial treatments for respiratory tract disease per 1,000 head days at risk.
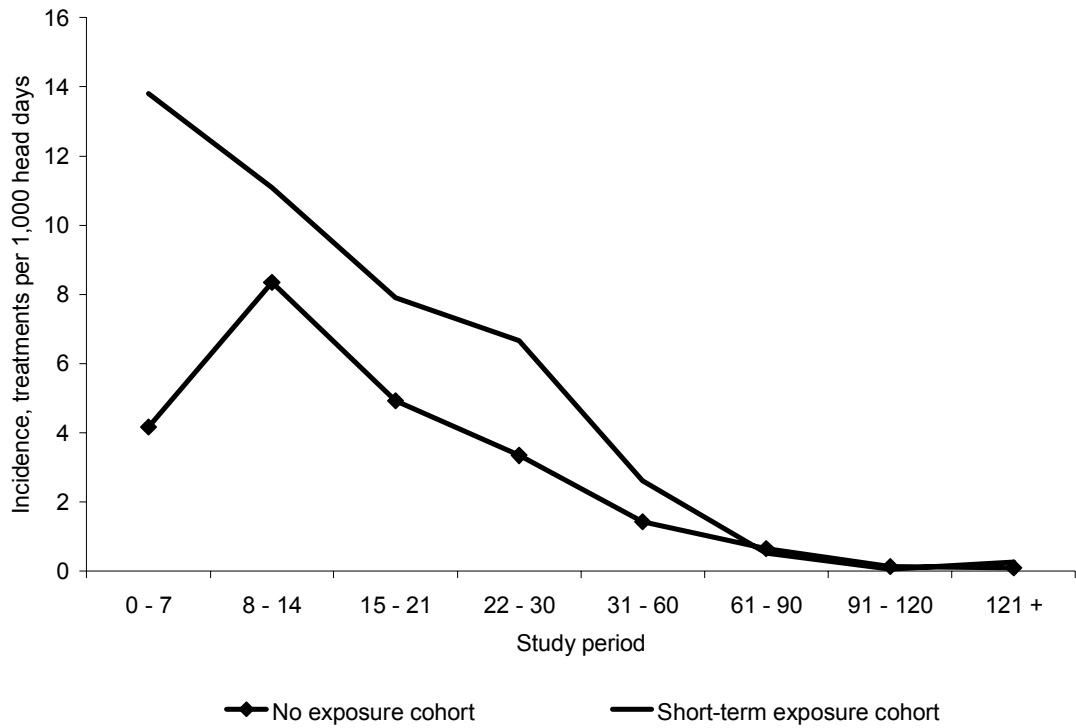
CHAPTER 3 - Testing and Removal of Feeder Calves Persistently Infected with Bovine Viral Diarrhea Virus at the Time of Feedlot Arrival and Outcome on Health, Performance and Carcass Characteristics

ABSTRACT

Twelve lots of auction derived steers totaling 1,577 head with an unknown health history (initial body weight 660 lb [300 kg]; 12 pens) were used to investigate testing and removal of feeder calves persistently-infected with bovine viral diarrhea virus (PI-BVDV) upon arrival at a single feedlot in central Kansas. Pens with a PI-BVDV calf on arrival were considered exposed and were compared to pens without a PI-BVDV calf on arrival. The exposed and non-exposed pens were followed from arrival through harvest to investigate the impact of exposure on health, performance, and carcass characteristics of feedlot cattle. A significant difference in the morbidity between exposed (2.3%) and non-exposed (7.2%) cattle was found ($P < 0.01$). No differences were found between the two groups for retreatment or mortality rates. Exposure to a PI-BVDV individual for less than 48 hours after arrival did not have an effect on performance parameters (final body weight, days-on-feed, average daily gain, dry matter intake, gain to feed, or cost of gain). There was an increased percentage of USDA yield grade 4 and 5 ($P = 0.01$) carcasses in the exposed group, but there were no other differences in carcass characteristics between groups.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an important pathogen of cattle, and infection can lead to a variety of adverse health outcomes such as enteritis, abortion, fetal malformations, and bovine respiratory disease (BRD).[7] The outcome of BVDV fetal infections in susceptible heifers and cows is dependent on the age of the fetus when exposed. Persistent infection in a calf develops when a susceptible fetus is exposed to a non-cytopathic BVDV during pregnancy at approximately 45 to 125 days of gestation.[8] Persistently infected animals are a continuous source of virus and can shed the virus in virtually all secretions and excretions, including nasal discharges, saliva, semen, urine, tears, milk, and, to a lesser extent, feces.[2,3,4,10]

Prevalence of persistently infected (PI) BVDV feeder cattle entering feedlots is estimated to be 0.3%.[7,12] During the feeding period, calves PI with BVDV tend to have lower growth rates and often die from mucosal disease.[9] Although few cattle PI with BVDV arrive at feedlots, the risk of initial treatment for BRD was 43% greater in cattle exposed to a PI calf.[7] Given the potential negative impact of exposure to a calf PI with BVDV, it may be advantageous to test newly arrived cattle. There are a number of tests available to practitioners; however, the BVDV antigen capture enzyme linked immunosorbent assay (ACE) is often used for the initial screening of feedlot cattle. A number of sample handling practices have been evaluated and found to have little impact on test sensitivity and specificity of ACE testing for BVDV.[6,11]

Effects of testing and removing PI cattle at revaccination (10 – 14 days on feed) were determined in a previous study.[12] No differences for mortality rates, retreatment rates, performance, or carcass characteristics were evident. Morbidity rates were different between the non-exposed and exposed groups (19% for non-exposed vs. 30% for exposed). Based on these

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findings, this trial was conducted to assess the impact of testing and removing PI cattle within two days after arrival.

MATERIALS AND METHODS

Cattle and sample collection

Twelve lots of auction-derived steers totaling 1,577 head with an unknown health history (initial BW 660 lb, ± 50.9; 299 kg ± 23.1) arrived at a commercial feedlot (one-time capacity of 12,000 animals) in central Kansas between March 2006 and October 2006. After arrival to the feedlot, cattle were placed in receiving pens and offered free choice hay and water. Cattle were processed after being allowed one hour of rest for every one hour of transport to the feedlot. All calves were processed within 24 hours of arrival. At processing, animals received a unique identification tag, administered doramectin,[a] a steroid growth implant,[c] and a modified-live vaccine containing bovine herpesvirus type 1, parainfluenza type 3, BVDV (Types 1 and 2), and bovine respiratory syncytial virus[b] at arrival. During initial processing, fresh skin (ear notch) specimens were collected and placed in phosphate buffered saline solution to be tested for BVDV antigen by ACE.

After initial processing, cattle were housed in 12 pens (range 62-302 animals/pen) and managed in accordance with routine feedlot practices. Ten to 14 days after initial processing the cattle received a second MLV vaccine[d] and a multivalent clostridial bacterin-toxoid.[e]

Animals that exhibited one or more clinical signs consistent with respiratory disease (depression, mucopurulent nasal discharge, increased respiratory rate and effort, and/or anorexia) were removed from the home pen for diagnosis. Animals with a rectal temperature greater than

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103.5°F (39.7°C) were treated with an antimicrobial. Additionally, all cattle in the feedlot were tested for PI-BVDV and removed if determined to be positive. All pens were maintained in accordance with standard feedlot practices. The first, second, and third antimicrobial treatments for BRD were tulathromycin,[f] florfenicol,[g] and ceftiofur sodium,[h] respectively.

Antigen Capture ELISA

Detection of BVDV antigen in skin specimens (ear notch) was performed using a commercial ACE kit.[i] Results were calculated by the following equation: standardized optical density (OD) = (raw OD of sample – raw OD of negative control)/(raw OD of positive control – raw OD of negative control). Samples with standardized OD values < 0.20 were considered negative, and those with OD values > 0.39 were considered positive. Samples with values from 0.2 to 0.39 were retested with detector reagents with or without antibody. Upon secondary analysis, no animals had values from 0.20 – 0.39. Animals that tested positive by antigen capture ELISA were removed from the pen, isolated, and retested 21 days later by immunohistochemistry (IHC) for confirmation of PI-BVDV status.

Assignment to Treatment Groups

All cattle that arrived at the feedlot were tested for PI-BVDV by ACE. If an animal tested positive for BVDV using ACE testing, it was removed from the home pen (range = 1 – 2 days-on-feed). Not all the pens that were tested contained a PI-BVDV animal. When an animal PI with BVDV was found in a pen, a pen with no PI-BVDV animals was identified. This created a pair of pens to be followed over time. Paired pens were similar with respect to initial weight,

date of arrival (same week), sex, and geographical origin. After a pair of pens was enrolled, the exposed and NE pens were followed through closeout and harvest.

Health and Performance Data

Feedlot data were collected from electronic records maintained at the feedlot. Data obtained from the closeout sheets included initial body weight (BW), final BW, days-on-feed, average daily gain (ADG), dry matter intake (DMI), gain to feed (G:F), and feed cost per pound of gain (COG).   Initial and final body weight was determined by the average weight of the lot at the time of arrival or harvest, respectively. Health data were recorded daily by trained feedlot personnel. The feedlot and pen riders were masked (blinded) from treatments. Pens were harvested based on visual appraisal as well as targeted harvest dates. Paired pens were harvested at approximately the same time (within the same week). Data retrieved from the animal health computer system[j] included respiratory morbidity rate, number of treatments, death loss, and treatment costs.

Statistical Analysis

Performance based data (ADG, DMI, initial BW, final BW, G:F, dressing percent, and COG) were analyzed as a single factor experiment using the general linear model of SAS release 9.1.3. Pen was the experimental unit. Non-parametric data (morbidity rates, retreatment rates, mortality rates, quality grade, and yield grade) were tested as binomial proportions using the Glimmix procedures in SAS. Percentages are reported in tables for animal health and carcass variables. The largest standard error of the least squares means is reported in the tables.

RESULTS

Five of the six pens that were considered positive on arrival had one PI animal on arrival. The sixth pen that was considered positive on arrival contained 4 PI animals. Following a positive ACE test, PI animals were removed and fed. The health and performance of the positive animals were not followed. Animal health and feedlot performance data are in Tables 3.1 and 3.2. A significant difference in the morbidity between exposed (2.3%) and non-exposed (7.2%) cattle was found ($P < 0.01$). There were no differences between groups for retreatment rate or mortality rate. There were no differences in ADG ($P \geq 0.28$), DMI, G:F or cost of gain between exposed and NE groups. There were no differences in dressing percent ($P = 0.55$) or quality grade ($P = 0.46$) of carcasses from the exposed and NE groups (Table 3.3). There were no differences for calculated yield grades 1 and 2 ($P = 0.46$) or yield grade 3 ($P = 0.26$) between different BVDV exposure groups of cattle; however, percentage of carcasses with a calculated yield grade of 4 and 5 was higher in cattle exposed to BVDV ($P = 0.01$).

DISCUSSION

In this study, cattle with no exposure to PI calves early in the receiving period had higher morbidity rates than the cattle exposed to a PI calf; however, morbidity rates for both groups of cattle were less than 8% (Table 3.1). In a previous study conducted at this facility, cattle with short-term exposure to a PI animal (tested at d 10 – 14 and removed d 13 – 18) had higher morbidity rates than cattle with no exposure (30% vs. 19%, respectively).[12] In a large pen study, Loneragan *et al* found that cattle within a pen that contained a PI animal were at slightly greater risk of BRD than non-exposed cattle(7.36% vs. 5.14%). A more profound impact was found when adjacent pens were included in their analysis. When adjacent pens were included in the

analysis, the incidence of treatment for respiratory disease was 43% greater.[7] In another large

pen study, Booker *et al* found no difference in the respiratory morbidity rate of cattle in pens that

contained a PI individual compared to those housed in pens without a PI animal; however, they

were not able to evaluate the health of adjacent pens. There was no difference in total mortality;

however, there was a difference in BVDV/enteritis mortality.[1] In a small pen study, Elam *et al*

found no differences in animal health within pens and adjacent pens that had short- or long-term

exposure to PI-BVDV animals.[5] In our current study, adjacent pens were not analyzed because

there were no fence-line water tanks in the feedlot. In addition, all groups were housed next to

pens that did not contain a PI animal at any point during the study.

Interestingly, there was a significant difference in the morbidity between cattle that had

no exposure to a PI-BVDV animal (7.2% morbidity) and those that were exposed (2.3%

morbidity rate; $P = 0.01$). In a longitudinal study at a custom cattle feeding operation in Iowa,

O'Connor *et al* reported that inclusion of a calf PI with BVDV in a pen was associated with

reduced disease risk for undifferentiated bovine respiratory tract disease (UBRTD) and chronic

disease (odds ratio < 1).[9] In that study, the authors reported a decrease of approximately 30% in

the risk of UBRTD in a pen containing a PI-BVDV calf compared with the risk of UBRTD in a

pen without a PI-BVDV calf. Furthermore, the mean cumulative incidence of morbidity

attributable to any disease during the feeding period was lowest in pens that contained cattle

from a single source with a PI-BVDV calf, compared with the mean cumulative incidence of

morbidity from pens containing cattle from a single source and no PI-BVDV calf.[9] This suggests

that exposure to a PI-BVDV calf at an early stage of development while experiencing minimal

stress may actually better prepare herdmates of PI calves for disease exposure upon feedlot entry,

compared to calves with no prior exposure to a PI animal.

In our current study we found no differences in the performance variables during the study period. Carcass quality was not affected and no differences were found for the majority of yield grade data; however, there was an increase in yield grade 4 and 5 carcasses in the exposed group. Results from the small pen study by Elam *et al* found no differences in final BW, DMI, ADG and G:F in calves with direct or adjacent exposure to a PI-BVDV calf, but they did find a tendency ($P \leq 0.12$) for PI exposed cattle to gain less through day 28. However, these differences were not noticeable by day 56. In a large pen study,[1] no significant differences in ADG or dry matter to gain ratio between PI pens and non-PI pens of cattle were found.

A limitation to the present study design was the lack of a treatment group that included pens that contained a PI-BVDV animal that was not removed after identification. This would have allowed for a greater understanding of the full impact that a calf PI with BVDV could have on health and performance measures under our experimental conditions. Future research efforts should be directed towards understanding the role of a PI-BVDV animal when it is not removed from the pen within commercial feeding systems.

CONCLUSIONS

Under the conditions of this study, there were no harmful outcomes when newly arrived feeder cattle were exposed to a PI animal for one to two days following feedlot entry.

Endnote

[a]Dectomax injectable, Pfizer Animal Health, New York, NY

[b]Arsenal 4.1, Novartis Animal Health, Greensboro, NC

[c]Component E-S, Vetlife, West Des Moines, IA

[d]Vista 5 SQ, Intervet, Millsboro, DE

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^eVision 7, Intervet, Millsoboro, DE

^fDraxxin, Pfizer Animal Health, New York, NY

^gNuflor, Schering Plough Animal Health, Union, NJ

^hExcenel, Pfizer Animal Health, New York, NY

ⁱIdexx Laboratories Inc., Westbrook, ME

^jWalco International, Amarillo, TX

^kSAS System for Windows 9.1.3, SAS Inst. Inc., Cary, NC

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Table 3.1. Health data from pens of feeder cattle that did (PI-pen) or did not (non-PI pen) have contact with a persistently infected (PI) calf on arrival to a commercial feedlot. Variables are least squares means expressed as percents.

Variable	PI pen	Non-PI pen	SEM	P-value
No. pens	6	6		
No. animals	909	668		
Initial weight (lb)	647	679	50.9	0.66
Initial BRD treatment (%)[1]	2.7	7.0	3.0	< 0.01
Retreatment rate (%)[2]	4.8	0.5	3.4	0.29
Mortality rate (%)[3]	1.6	1.2	0.5	0.41

[1]Initial bovine respiratory disease (BRD) treatment rate is the number of animals treated for BRD divided by the number of animals placed in the study.

[2]Retreatment rate is the number of cattle treated a second or third time for BRD divided by the number of animals first treated for BRD.

[3]Mortality rate is the number of cattle that died of BRD divided by the number of animals placed in the study.

Table 3.2. Performance data from pens of feeder cattle that did (PI pen) or did not (non-PI pen) have contact with a persistently infected (PI) calf on arrival to a commercial feedlot.   Variables presented are least squares means.

Variable	PI Pen	Non-PI Pen	SEM	P-value
Initial BW, lb	647	679	50.9	0.66
Final BW, lb	1216	1147	36.9	0.22
ADG, lb/day	2.6	2.4	0.16	0.28
DMI, lb/day	20.1	19.3	1.3	0.67
F:G	7.7	8.1	0.38	0.43
COG, $/lb	0.86	0.87	0.06	0.92

Table 3.3. Least squares means carcass characteristics from pens of feeder cattle that did (PI pen) or did not (non-PI pen) have contact with a persistently infected (PI) calf on arrival to a commercial feedlot.

Variable	PI pen	Non-PI pen	SEM	P-value
Dressing percent (%)	62.8	62.4	0.52	0.55
USDA Quality Grade				
Choice/Prime (%)	81.7	81.4	6.1	0.46
Select or Other (%)	18.3	18.8	6.1	0.46
USDA Yield Grade				
1 and 2 (%)	26	31	4.4	0.46
3 (%)	59.8	62	3.8	0.26
4 and 5 (%)	14.2	7	3.2	0.01

CHAPTER 4 - The Survival of Bovine Viral Diarrhea Virus on Materials Associated with Livestock Production

ABSTRACT

Cattle that are persistently infected with bovine viral diarrhea virus (BVDV) serve as the predominant reservoir of virus for in-contact cattle. Persistently infected cattle are processed and housed within facilities that are shared with naïve cattle. Furthermore, the handlers of PI-BVDV cattle may have multiple interactions with naïve cattle and may exacerbate transmission of the virus. To date, there are limited published reports of the survival of BVDV on materials commonly associated with livestock production. Through the use of a controlled environment, we characterized the survival of a non-cytopathic, Type 1b BVDV isolate from a persistently infected animal on materials commonly associated with livestock production that may serve as fomites for the transmission of BVDV.

INTRODUCTION

Transmission of pathogens from infected to susceptible hosts occurs by direct or indirect means. Indirect transmission may take place via water, food, air or contaminated fomites. A critical factor in the indirect transmission of any pathogen is its ability to survive in the environment. Excretion of large amounts of respiratory viruses in excretions or secretions of infected animals may lead to environmental contamination. Viral survival for any length of time on environmental surfaces and fomites may help their transmission to susceptible hosts[8].

Transmission of bovine viral diarrhea virus (BVDV) is thought to be through direct contact via oral and nasal routes; however, aersolization has also been reported [17].

Cattle persistently infected with BVDV can shed BVDV into the environment through secretions and excretions, including nasal discharges, saliva, semen, urine, tears, and to a lesser extent, feces. Work by Brock et al. focused on characterizing the viremia associated with PI-BVDV calves. From their published report, all persistently infected calves in the study were viremic by 42 days of age. Further characterization of 7 mature PI-BVDV animals found a range of outcomes over time. The majority of the animals, 5 of 7, declined in viremia by one 10-fold dilution over a two year period. Of the two remaining animals in the study, one had a 10 fold increase in the level of viremia during the 2 year period, while the viremia of the other animal fell below detectable levels by serum virus isolation using cell culture[1].

The manifestations of BVDV are varied and are of economical concerns to producers at all levels of production as it plays a role in bovine respiratory tract disease and immunosuppression of stocker and feedlot cattle[4]. At the cow-calf level, the outcome of BVDV fetal infections in susceptible heifers and cows is dependent on the age of the fetus when exposed. Infection can result in abortion, stillbirths, congenital malformations and birth of persistently infected (PI) calves. Persistent infection in a calf develops when a susceptible heifer or cow is exposed to a noncytopathic BVDV during pregnancy at approximately 45 to 125 days of gestation[6]. The prevalence of persistently infected BVDV feeder cattle entering feedlots is estimated to be 0.3 %[5]. PI cattle are important sources of virus for animals in direct or close contact. In a recent study the risk of initial treatment for bovine respiratory disease (BRD) was 43 % greater in cattle exposed to a PI calf[5]. Of the various biotypes, genotypes and subtypes of BVDV, Type 1b was found to be the most prevalent type isolated from persistently infected

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BVDV cattle entering a feedlot[2]. Persistently infected calves tend to have lower growth rates and often die from classic mucosal disease or other diseases during the feeding period[9].

Survival of BVDV in the environment away from the host is important to its ability to spread. In general, pestiviruses have very limited ability to maintain their infectivity outside the host. BVDV rapidly loses infectivity after contact with organic solvents and pH outside the range of 6.7 to 9.3[13]. Its sensitivity to low pH increases as environmental temperatures increase from 4°C to 37°C[13]. Both biotypes are equally sensitive to temperature and pH. Other pestiviruses are readily inactivated by heat, desiccation, ultraviolet light, detergents, and organic solvents[14]. Experimentally, it has been shown that BVDV can be spread by fomites. Specifically, rectal sleeves, intravenous needles, and nose tongs have been implicated[15, 16]. To date there are no published reports on the survival of bovine viral diarrhea virus on other important materials associated with livestock production. Therefore, the objective of this study was to assess the survival of a non-cytopathic, Type 1b, BVDV on materials commonly used or encountered in livestock production.

MATERIALS AND METHODS

Virus

The BVDV strain CA0401186a was used for testing survival on fomites. The CA0401186 strain was obtained from Dr. Julia Ridpath (NADC, Ames, IA). Strain CA0401186a was isolated from the tissues of a PI calf submitted to the National Animal Disease Center (NADC) by diagnosticians from the Tulare Laboratory of the California Animal Health and Food Safety Laboratory. The calf was one of 24 premature calves with brain and skeletal deformities born to heifers in one herd. It was one of seven calves from the outbreak that suffered from brachygnathism. CA0401186 has been characterized as non-cytopathic based on

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growth characteristics in Madin-Darby bovine kidney (MDBK) cells. Based on comparison of sequences from the 5' UTR, CA0401186a has been segregated to the BVDV 1b subgenotype (Ridpath et al.).

Preparation of Viral Suspensions

Virus was propagated in bovine turbinate (BT) cells to produce a single lot of virus for the study. After propagation of the single lot of virus, it was aliquoted, frozen, and stored at $-80°C$ for future use. Two vials of virus, the first and last aliquot of the single lot, were used for determination of the titer. After the titer was determined, 2 working concentrations of virus were made for application to fomites. The first was a 2x 4.1 $\log_{10}$ median tissue culture infective dose (TCID$_{50}$)/ml, and the second was a 3x 4.1 $\log_{10}$ TCID$_{50}$/ml solutions. Each respective working concentration of virus was diluted in either phosphate buffered saline (PBS; pH=7.4; Cellgro, Manassas, VA) or a 20 % mucus/PBS solution (pH = 7.4).

Preparation of Mucus

A synthetic mucus was prepared that contained 1 g guar gum, 0.5 g dried type II mucin from porcine stomach, 0.26 g monobasic potassium phosphate, 1.57 g dibasic potassium phosphate, 1 ml 0.1 M sodium borate solution, and 96.2 g distilled water[10]. All chemicals were purchased from Sigma-Aldrich. The mixing process involved slow dissolution of guar gum into 90% of the distilled water using a standard magnetic stir bar. After complete hydration, noted by a visual increase of the solution's viscosity, mucin was slowly added. While the mucin mixed, the buffer salts were dissolved into the remaining 10% of the distilled water and then slowly

added to the viscous solution. Once mixed well, the borate solution was added to the solution. The synthetic mucus was then sterilized by autoclaving.

Preparation and Inoculation of Fomite Samples

All fomites were replicated three times. Virus was diluted in either 20 % mucus PBS or PBS and applied to the fomite. For this trial temperature and humidity were monitored. For the three independent trials, the range of the room temperature was 77 – 82°F and the range of relative humidity was 22 – 27 %.

Paper (computer print paper), latex glove material, and clothing (a 100 % cotton t-shirt, denim jeans)

One centimeter squares were cut from each of the materials. They were sterilized by autoclaving, and after drying each piece was placed in a separate well of a 24 well culture plate purchased through (Greiner Bio-One North America, Monroe, NC). A total of 40 µl, in two separate 20 µl aliquots, of a 2x 4.1 log 10 $TCID_{50}$/ml virus suspension was added to each fomite.

Wood (pine, not treated with preservative), rubber (Wellington boot), metal (galvanized vs. enameled),

One centimeter squares were cut, autoclaved and allowed to dry. Each fomite was placed in a separate well of a 6 well culture plate (Greiner Bio-One North America, Monroe, NC). A total of 40 µl, in two separate 20 µl aliquots, of a 2x 4.1 log 10 $TCID_{50}$/ml virus suspension was added to each fomite.

Mineral, Salt, and Molasses Urea Blocks

An approximate 0.5 – 1.0 cubic centimeter piece of mineral, salt, or of a molasses urea lick were isolated from the main block or lick using a hammer and chisel. A total of 40 µl, in two separate 20 µl aliquots, of a 2x 4.1 log 10 $TCID_{50}$/ml virus suspension was added to each fomite.

Total Mixed Ration and Pen floor soil/manure mix

Aliquots of 0.3 g of each material were weighed, placed into a 2.0 ml centrifuge tube (Continental Lab Products, San Diego, CA), autoclaved, and allowed to dry prior to use. A total of 40 µl, in one aliquot, of a 3x 4.1 log 10 $TCID_{50}$/ml virus suspension was added to each fomite.

Water & Phosphate Buffered Saline(PBS)

Distilled water (Cellgro, Manassas, VA) and PBS (Cellgro, Manassas, VA) were autoclaved. After autoclaving, 40 µl of a 2x 4.1 log 10 $TCID_{50}$/ml virus suspension was added.

Collection of Samples

Samples were collected immediately after the virus suspension was applied to the fomite (0 hour). Additionally, samples were collected at 1, 2, 4, 6, 8, 24, 48, and 96 hours. Fomites were eluted with 1.0 ml (paper, latex, cotton t-shirt, denim, rubber, metal (galvanized and enameled), wood, mineral block, salt block, molasses urea lick) or 1.5 ml (feed and dirt) of virus maintenance media. Virus maintenance media (VMM) contained 480 ml Minimum Essential Medium Eagle (MEM) supplemented with Earle's Salts (Cellgro, Manassas, VA), 10 ml horse serum (Atlanta Biologicals, Lawrenceville, GA) 5 ml of a stock antibiotic/antimycotic (10,000

U/ml penicillin; 10,000 ug/ml streptomycin; 25 ug/ml amphotericin B; Atlanta Biologicals,

Lawrenceville, GA), and 5 ml of a stock 200 mM L-glutamine (Atlanta Biologicals,

Lawrenceville, GA).

Virus Detection

Virus isolation in BT cells was attempted on every sample. The propagation and

detection has been described previously (Saliki et al. 1997). Briefly, BT cells were seeded two

days prior to use into 96 well cell culture microtiter (Greiner Bio-One North America, Monroe,

NC). A total of 100 µl of the eluted sample was added in triplicate to each respective well.

After plating all fomite samples, 100 µl of virus maintenance media was added to all wells.

After a four day incubation at 37°C in 5 % CO_2, the plates were frozen at – 80 °C and thawed for

a total of three consecutive times. After the last freeze-thaw, 100 µl of the supernatant for each

respective sample and 100 µl of virus maintenance media were transferred to a two day old 96

well cell culture microtiter plate previously seeded with BT cells. This methodology was

completed an additional two times, thus creating a three pass virus isolation. After the third pass,

the media was removed and the plates were dried at room temperature. After thoroughly drying,

the monolayers were fixed in a 20 % acetone PBS solution for 10 minutes and then allowed to air

dry. The fixed monolayers were incubated with a 1:400 dilution of the BVDV monoclonal

antibody 20.10.6 in PBS containing 0.05% Tween 20 (Sigma, St. Louis, MO) for 45 minutes at

37°C. Following four washes with PBS containing 0.05% Tween 20, the plates were incubated

with a 1:400 dilution of biotinylated rabbit anti-mouse IgG (Invitrogen, Carlsbad, CA) in binding

buffer supplemented with 60% chicken serum (Atlanta Biologicals, Lawrenceville, GA) for 45

minutes at 37°C. Following a wash step as above, a 1:1000 dilution of peroxidase-conjugated

streptavidin (Invitrogen, Carlsbad, CA) was added and the plate was incubated for 45 minutes at 37°C. After a final wash, substrate containing 280 µg of 3-amino-9-ethylcarbazole (Invitrogen, Carlsbad, CA) per ml and 0.01% hydrogen peroxide in 50 mM acetate buffer (pH 5.0) was added and the mixture was incubated at 37°C in the dark. A positive reaction was recorded when there was an appearance of a red intracellular precipitate after 30 minutes. Test samples were considered positive if they produced, in at least one well, distinct cytoplasmic staining. If there was no color development, the sample was deemed negative.

Data Analysis

Data was analyzed using the logistic procedure in STATA 10.1™ (College Station, TX). The data was transformed to model the effect of time on the likelihood of recovering virus at each time point using logistic regression. In order to achieve model stability, all of the 96 hour time-point observations were dropped because of lack of variability in the data (predicted no virus present perfectly). Also, observations for the fomites: cotton t-shirt, denim, total mixed ration mineral lick, salt lick, and molasses urea lick were dropped as they perfectly predicted that no virus was found. Therefore, the logistic model was derived from a total of 486 observations. Due to the inherent study design, only the first order model was explored. Therefore, no interactions were statistically analyzed. After achieving a satisfactory model, the PRVALUE procedure in STATA™ (College Station, TX) was used to achieve model adjusted risk estimates and corresponding 95% confidence intervals for the risk of finding virus present at each time point for each fomite (metal, wood, rubber, etc…) and treatment (PBS & mucus). This data was then organized and graphically shown using error bars at each data point to give a visual

representation of the data and the relative significance of any differences between treatments at each time point.

RESULTS

When controlling for treatment, there were visually discernable differences in survival of BVDV strain CA040118a between fomite (Figure 4.1). The probability of virus being present decreased as time of incubation increased. The aqueous solutions (water and PBS), most non-porous materials (latex and enameled metal), and one porous material (paper) tended to have the highest probability of recovering virus. One non-porous material (galvanized metal) and two porous materials (soil and pine) tended to have the lowest probability of recovering virus.

Survival on paper

On paper, BVDV strain CA0401186a tended to have a higher risk of being isolated in mucus than PBS (Figure 4.2; P = 0.052). The risk of virus being present decreased as time of incubation increased for both mucus viral suspension (MV) and PBS viral suspension (PV). Visually there was a significant difference between MV and PV at 2, 4, and 6 hours post incubation based on non-overlapping error bars. The treatment*time interaction for this fomite was examined and found to not be significant (P = 0.076). Characterization of survival revealed there was a 94 % chance that virus would be present at 1 hour for MV and a 62 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was only a 39 % chance that virus would be present in MV group compared to 7 % in the PV group. By 48 hours post incubation, there was less than a 7 % chance that virus would be present in either group. However, these differences between treatments were not statistically significant.

Survival on Latex

On latex, BVDV strain CA0401186a generally survived longer in mucus than PBS (Figure 4.3; P = 0.075). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was a significant difference between MV and PV at 4 and 6 hours post incubation based on non-overlapping error bars. The treatment*time interaction could not be determined due to co-linearity. Characterization of survival revealed there was a 98 % chance that virus would be present at 1 hour for the MV and an 85 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was a 70 % chance that virus would be present in MV group compared to 20 % in the PV group. By 48 hours post incubation, there was a 20 % chance that virus would be present in the MV group and less than 3 % in the PV group. With the exception of 6 hours post incubation, there was no difference in survival between treatments for 1 and 48 hours post incubation.

Survival on Rubber

On rubber from a wellington boot, BVDV strain CA0401186a generally survived longer in mucus than PBS (Figure 4.4; P = 0.638). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was a significant difference between MV and PV at 1, 2, and 4 hours post incubation based on non-overlapping error bars. The treatment*time interaction for this fomite was found to be significant (P = 0.057) with the earlier time points of 1, 2, and 4 hours being different than the later time points. Characterization of survival revealed there was an 89 % chance that virus would be present at 1 hour for MV and a 46 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was a 25 % chance that virus would be present in MV compared to 4 % in the PV. By 48 hours post

incubation, there was less than a 4 % chance of survival from either group. With the exception of 1 hour post incubation, there was no difference in survival between treatments for 6 and 48 hours post incubation.

Survival on Pine (not treated with preservative)

The survival of BVDV strain CA0401186a was reduced when applied to pine that was not treated with preservatives (Figure 4.5). The risk of virus being present decreased as time of incubation increased for both MV and PV. There were no statistical differences between MV and PV based on non-overlapping error bars. Characterization of survival revealed there was a 31 % chance that virus would be present at 1 hour for MV and a 5 % chance that virus would be present at 1 hour for PV. At 6 hours there was less than a 2 % chance that virus would be present for either group. At 8 hours and beyond, there was minimal chance (< 1 %) of the virus being present. However, these differences between treatments were not statistically significant.

Survival on Galvanized Metal

The survival of BVDV strain CA0401186a was reduced when applied to galvanized metal (Figure 4.6). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was no significant difference between MV and PV based on non-overlapping error bars. Characterization of survival revealed there was a 22 % chance that virus would be present at 1 hour for MV and a 3 % chance that virus would be present at 1 hour for PV. At 6 hours there was less than a 2 % chance that virus would be present for either group. At 8 hours and beyond, there was minimal chance (< 1%) of the virus being present. However, these differences between treatments were not statistically significant.

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Survival on Enameled Metal

On enameled metal, BVDV strain CA0401186a generally survived longer in mucus than PBS (Figure 4.7; P < 0.05). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was a significant difference between MV and PV at 1, 2, and 4 hours post incubation based on non-overlapping error bars. The treatment*time interaction could not be determined due to co-linearity. Unlike the lack of survival on galvanized metal, there was a 89 % chance that virus would be present at 1 hour for MV and a 46 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was a 25 % chance that virus would be present in the MV group compared to 4 % in the PV group. By 48 hours post incubation, there was less than a 4 % chance of survival from either group. With the exception of 1 hour post incubation, there was no difference in survival between treatments for 6 and 48 hours post incubation.

Survival in Soil

The survival of BVDV strain CA0401186a was reduced when applied to soil (Figure 4.8). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was no significant difference between MV and PV based on non-overlapping error bars. A treatment*time interaction could not be determined as MV treatment predicted failure (no virus) for the majority of time points. Characterization of survival revealed there was a 48 % chance that virus would be present at 1 hour for MV and roughly 9 % chance that virus would be present at 1 hour for PV. At 6 hours there was less than 4 % chance that virus would be present for either group. At 8 hours and beyond, there was minimal chance (< 1 %) of the

virus being present. However, these differences between treatments were not statistically significant.

Survival in Phosphate Buffered Saline

When added to PBS, BVDV strain CA0401186a generally survived longer in mucus than PBS (Figure 4.9; P = 0.052). The risk of virus being present decreased as time of incubation increased for both MV and PV. The treatment*time interaction for PBS was not statistically significant (P = 0.12). Visually there was a significant difference between MV and PV at 2 and 4 hours post incubation based on non-overlapping error bars. Characterization of survival revealed there was a 95 % chance that virus would be present at 1 hour for MV and a 66 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was roughly a 43 % chance that virus would be present in the MV group compared to less than 8 % in the PV group. By 48 hours post incubation, there was less than an 8% chance of survival from either group. However, these differences between treatments were not statistically significant.

Survival in Water

When added to water, BVDV strain CA0401186a generally survived longer in mucus than PBS (Figure 4.10). The risk of virus being present decreased as time of incubation increased for both MV and PV. Visually there was a significant difference between MV and PV at 4 and 6 hours post incubation based on non-overlapping error bars. Characterization of survival revealed there was a 98 % chance that virus would be present at 1 hour for MV and an 82 % chance that virus would be present at 1 hour for PV. At 6 hours post incubation, there was a 64 % chance that virus would be present in the MV group compared to 17 % in the PV group.

By 48 hours post incubation, there was a 16 % chance that virus would be present in MV group compared to 2 % in the PV group. With the exception of 6 hours post incubation, there was no difference in survival between treatments for 1 and 48 hours post incubation.

Other Fomites

Data for the cotton t-shirt, denim, total mixed ration, mineral lick, salt lick, and molasses urea lick were not included in the analysis. No virus was recovered from the denim and cotton t-shirt elutions. For the total mixed ration, for one replicate, we recovered virus for up to 8 hours post application; however, all other replicates were negative. For the molasses urea lick in all three replicates, there was contamination, which invalidated the analysis. Lastly, the mineral and salt licks were negative at all time points.

DISCUSSION

In previous fomites trials, the experimental design focused on identifying a PI animal and then exposing the desired fomite (nose tongs, rectal sleeve, etc…) to the PI animal and using the exposed fomite for exposure to the naïve animal[15, 16]. Response variables in those trials consisted of clinical signs, virus isolation, and serum neutralization titers. For this trial, in a laboratory setting, we applied the virus to the fomite and then eluted the fomite at desired time points to determine if virus could be recovered. The objective was to determine the survival of a non-cytopathic, Type 1 BVDV on materials commonly used or encountered in livestock production. We chose a non-cytopathic biotype of BVDV because it is the biotype that is associated with the development of PI BVDV cattle. We chose to use the Type 1b genotype of BVDV because it was the genotype most often recovered from newly arrived cattle that were

found to be PI on arrival to a feedlot[3]. In addition to selecting a ncp, Type 1b BVDV, the isolate we selected had a clinical history consistent with the ability to produce PI animals with developmental anomalies. The concentration of the virus applied to the fomite was based on a previous publication[18]. Grooms et al. used PI cows as a source of continual exposure of BVDV in a commercial fetal protection vaccine trial. In that trial, BVDV was isolated from nasal swab specimens obtained from the PI cows. Virus titer for each PI cow (n = 3) in the trial ranged from 1.7 to 4.5 $\log_{10}$ $CCID_{50}$.

Two suspensions of virus were produced. Virus was diluted in standard PBS or virus was diluted in a 20 % synthetic mucus PBS (pH 7.4). The use of a synthetic mucus material in PBS was an attempt to achieve a reasonable system to test the survival of virus in a manner that is similar to natural exposure via nasal secretions. The pH for the solutions was determined from unpublished data which measured the pH of bovine nasal mucus at the Great Plains Veterinary Education Center, Clay Center, NE (personal communication). Prior to selecting the 20 % dilutional factor for the synthetic mucus, several dilutions were made (5, 10, 20, 50, 75, and 100 %). The 20 % dilutional factor was selected based on a subjective viscosity and consistency and an objective drying time. For the 20 % dilution factor the consistency and viscosity was most similar to that of bovine nasal mucus. The time from application of the MV to the fomite until the fomite was dry was approximately 30 minutes. For isolation of the virus from the fomites, it was necessary to put the samples into an in vitro cell culture system. Thus, the synthetic mucus was preferred over actual bovine mucus because of sterility. For this trial temperature and humidity were monitored. For the three independent trials, the range of the room temperature was 77 – 82°F and the range of relative humidity was 22 – 27 %.

Our method of virus detection was three (3) pass virus isolation assay. We chose this method due to increased sensitivity. In theory, the assay works by testing the initial sample, then after a defined time in cell culture, the cells and supernatants are frozen at - 80°C and thawed at room temperature, the freeze-thaw cycle continues two additional times. After the last freeze thaw cycle, the supernatant is harvested and added to the culture media of newly plated cells and the entire process is completed two additional times until there has been three passes in cell culture. Therefore, even one virion has the ability to multiply and be sufficient for detection. A 3-pass virus isolation assay not only allows us to detect virus, but it also allows for the detection of viable virus. For polymerase chain reaction (PCR), the target for detecting the presence or absence of virus is deoxyribose nucleic acid (DNA). Unfortunately, the mere presence of DNA does not allow for the detection of viable virus. Conceivably, a real time-polymerase chain reaction (RT-PCR) would allow for quantification of virus and then an inference could possibly be made that replication may have occurred based on the number of viral transcripts.

Data for the cotton t-shirt, denim, total mixed ration, mineral lick, salt lick, and molasses urea lick were not included in the analysis. No virus was recovered from the denim and cotton t-shirt elutions. For the total mixed ration, for one replicate we recovered virus for up to 8 hours post application; however, all other replicates were negative. For the molasses urea lick in all three replicates there was contamination, which invalidated the analysis. Lastly, the mineral and salt licks were negative at all time points. Interestingly, the tested cell monolayer was confluent and appeared healthy for the mineral and salt lick samples. One possible reason for the negative results may be attributed to a change in the osmolality in the cell culture media. Dialysis of the elutions prior to plating may have been beneficial to remove the salts from the elutions prior to

testing. Another possible consideration would be testing the elutions by PCR to determine if an elution was positive for virus.

The absorbency of the materials may have some effect on the survival of BVDV. On absorbent materials, virus suspensions dried very quickly and this may have influenced the survival on these materials. When controlling for treatment, there was a discernable visual difference in the survival between non-absorbent materials (enameled metal, rubber boot, and latex glove) and absorbent materials (paper, cotton, soil, and pine). Pine, which had one of the shortest survival times, was likely the most absorbent. This may have had an effect on the sampling, making it more difficult to recover virus that had been absorbed. The thickness of the pine may have hindered recovery of virus, since for paper, which had a higher chance of recovery, the elution volume was capable of penetrating the entire material and being recovered easily for testing.

The virus survived for the longest times when tested on latex and in PBS and water. Virus survived the longest when in mucus and when applied to water. This is an interesting finding when considering livestock watering systems. These systems can be used to provide drinking water to a number of cattle at any one time. Thus, if a PI animal was present, and using a shared water source, it is conceivable that virus may persist and the water source would serve as a reservoir of virus for other cattle. This scenario should also be assumed for acute BVDV infections as well.

One interesting finding was the result obtained from the galvanized metal. The importance of this result is not clear. The process of galvanization is a metallurgical process that is used to coat steel or iron with zinc. The reason this is done is to prevent rust from occurring by taking advantage of the relative corrosion resistance of zinc. The galvanized metal was

washed and autoclaved prior to use, so if the manufacturing process had introduced noxious chemicals they potentially would have been removed prior to our use. Potentially, the application of zinc has an anti-viral or anti-viral survival effect.

Based on the findings of this study, the addition of a biosecurity plan for working with cattle is important and should be tailored accordingly. The practice of washing boots, changing gloves, disinfecting livestock chutes and other equipment, and cleaning water tanks are important mechanisms that can mitigate the risk of virus transfer from fomites.

CONCLUSIONS

Under the conditions of this study, a ncp, Type 1b, BVDV was capable of surviving after application to various fomites. When in the presence of mucus, BVDV was protected from degradation for longer periods of time then when not in the presence of mucus.

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Figure 4.1. Probability of virus being present at each time point post inoculation for each fomite controlling for treatment. Each line represents the average of all six replicates (3 PBS and 3 mucus) at each time point.
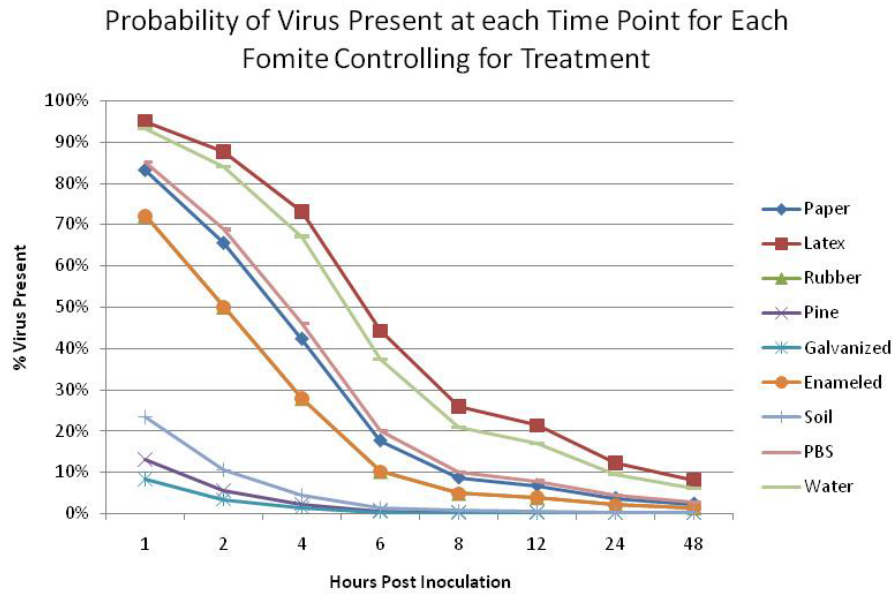
Figure 4.2. Model adjusted risk estimates of virus being present on paper. Based on three replicates at each time point for each treatment.

Risk of Virus Present at each Time Point for Paper
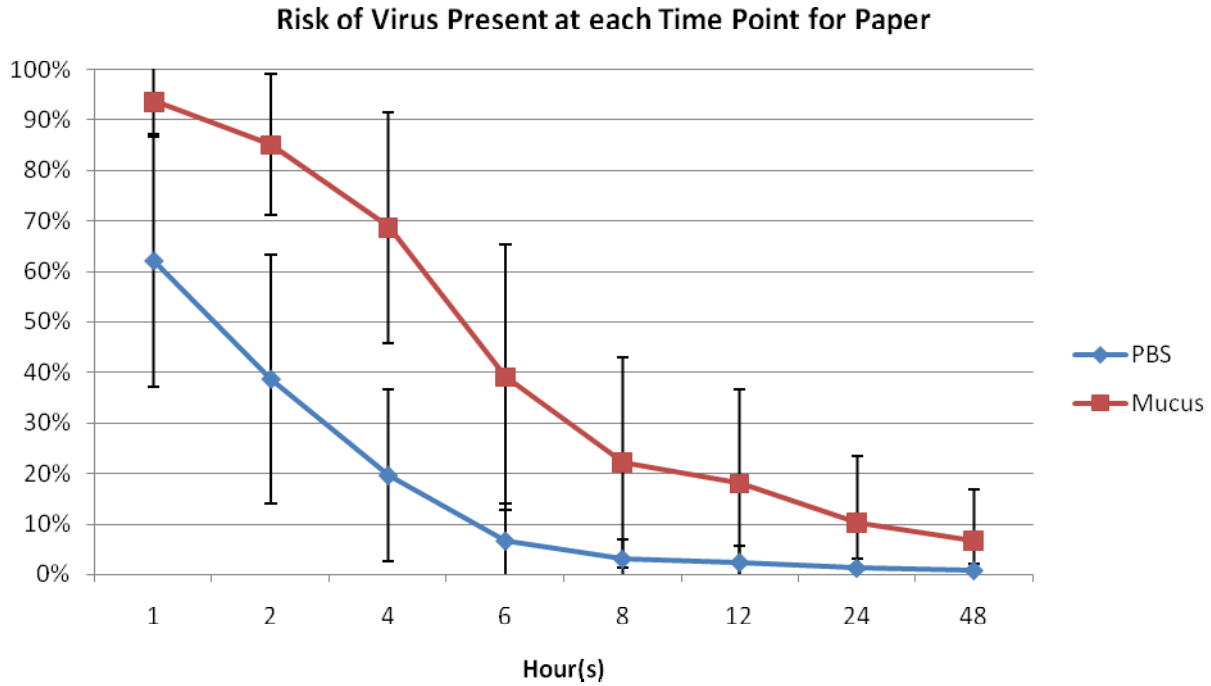
Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Paper	1	PBS	62.06%	37.05%	87.06%
Paper	2	PBS	38.66%	14.00%	63.32%
Paper	4	PBS	19.57%	2.62%	36.53%
Paper	6	PBS	6.63%	-0.78%	14.05%
Paper	8	PBS	3.05%	-0.90%	6.99%
Paper	12	PBS	2.37%	-0.85%	5.60%
Paper	24	PBS	1.24%	-0.70%	3.19%
Paper	48	PBS	0.77%	-0.60%	2.14%
Paper	1	Mucus	93.65%	86.70%	100.61%
Paper	2	Mucus	85.04%	71.04%	99.04%
Paper	4	Mucus	68.70%	45.82%	91.59%
Paper	6	Mucus	39.05%	12.89%	65.22%
Paper	8	Mucus	22.08%	1.27%	42.89%
Paper	12	Mucus	17.97%	-0.66%	36.60%
Paper	24	Mucus	10.19%	-3.16%	23.53%
Paper	48	Mucus	6.57%	-3.66%	16.81%

Figure 4.3. Model adjusted risk estimates of virus being present on latex. Based on three replicates at each time point for each treatment.

Risk of Virus Present at each Time Point for Latex

Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
latex	1	PBS	85.83%	72.51%	99.14%
latex	2	PBS	70.01%	48.00%	92.01%
latex	4	PBS	47.40%	21.19%	73.61%
latex	6	PBS	20.83%	2.13%	39.53%
latex	8	PBS	10.42%	-1.18%	22.02%
latex	12	PBS	8.25%	-1.53%	18.04%
latex	24	PBS	4.45%	-1.82%	10.72%
latex	48	PBS	2.81%	-1.76%	7.38%
latex	1	Mucus	98.20%	95.99%	100.42%
latex	2	Mucus	95.46%	90.41%	100.52%
latex	4	Mucus	89.05%	78.30%	99.70%
latex	6	Mucus	70.35%	47.54%	93.17%
latex	8	Mucus	51.20%	22.07%	80.34%
latex	12	Mucus	44.79%	14.89%	74.70%
latex	24	Mucus	29.58%	0.70%	58.46%
latex	48	Mucus	20.67%	-5.44%	46.77%

Figure 4.4. Model adjusted risk estimates of virus being present on rubber. Based on three replicates at each time point for each treatment.

Risk of Virus Present at each Time Point for Rubber Boot

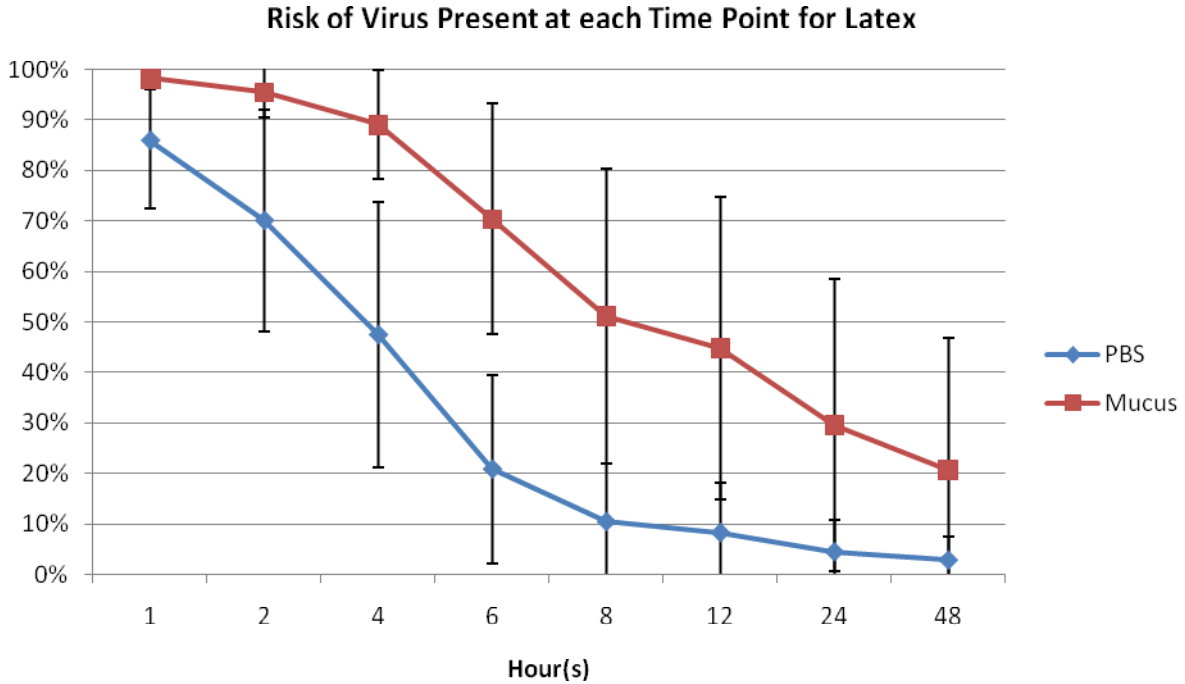| Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Rubber	1	PBS	46.28%	19.54%	73.02%
Rubber	2	PBS	24.93%	4.86%	44.99%
Rubber	4	PBS	11.36%	0.04%	22.69%
Rubber	6	PBS	3.61%	-0.79%	8.01%
Rubber	8	PBS	1.63%	-0.63%	3.88%
Rubber	12	PBS	1.26%	-0.56%	3.09%
Rubber	24	PBS	0.66%	-0.42%	1.74%
Rubber	48	PBS	0.41%	-0.34%	1.16%
Rubber	1	Mucus	88.60%	77.01%	100.18%
Rubber	2	Mucus	74.97%	54.44%	95.50%
Rubber	4	Mucus	53.63%	26.90%	80.35%
Rubber	6	Mucus	25.24%	3.78%	46.70%
Rubber	8	Mucus	12.99%	-1.20%	27.18%
Rubber	12	Mucus	10.35%	-1.80%	22.50%
Rubber	24	Mucus	5.64%	-2.35%	13.63%
Rubber	48	Mucus	3.57%	-2.30%	9.45%

Figure 4.5. Model adjusted risk estimates of virus being present on pine. Based on three replicates at each time point for each treatment.

Risk of Virus Present at each Time Point for Pine
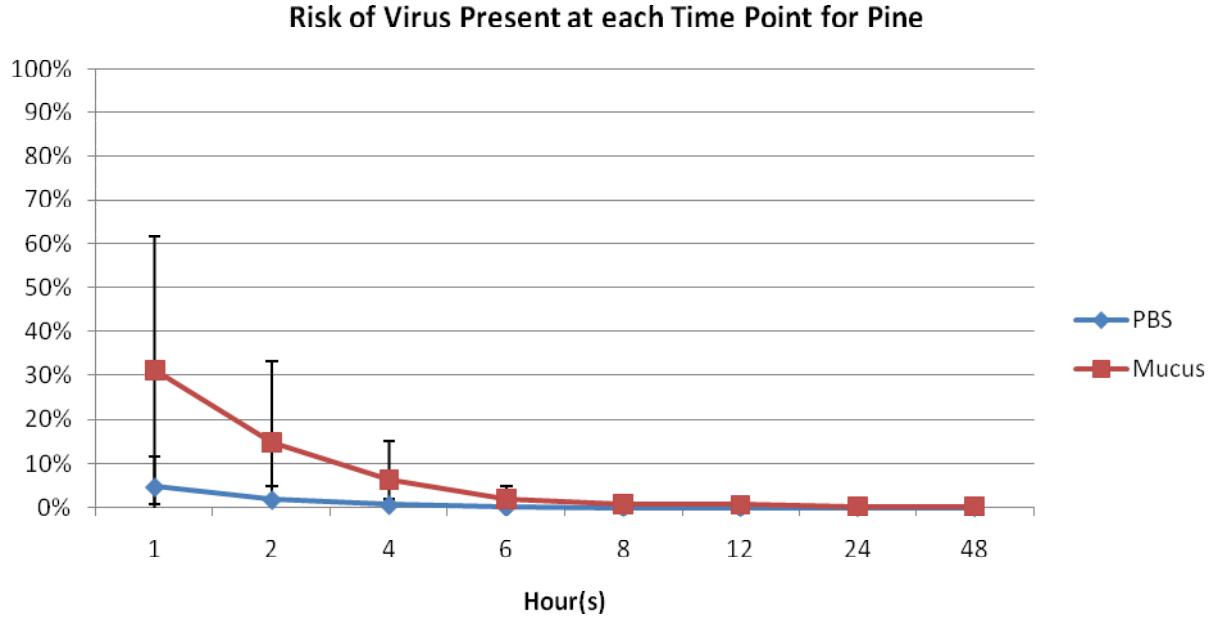
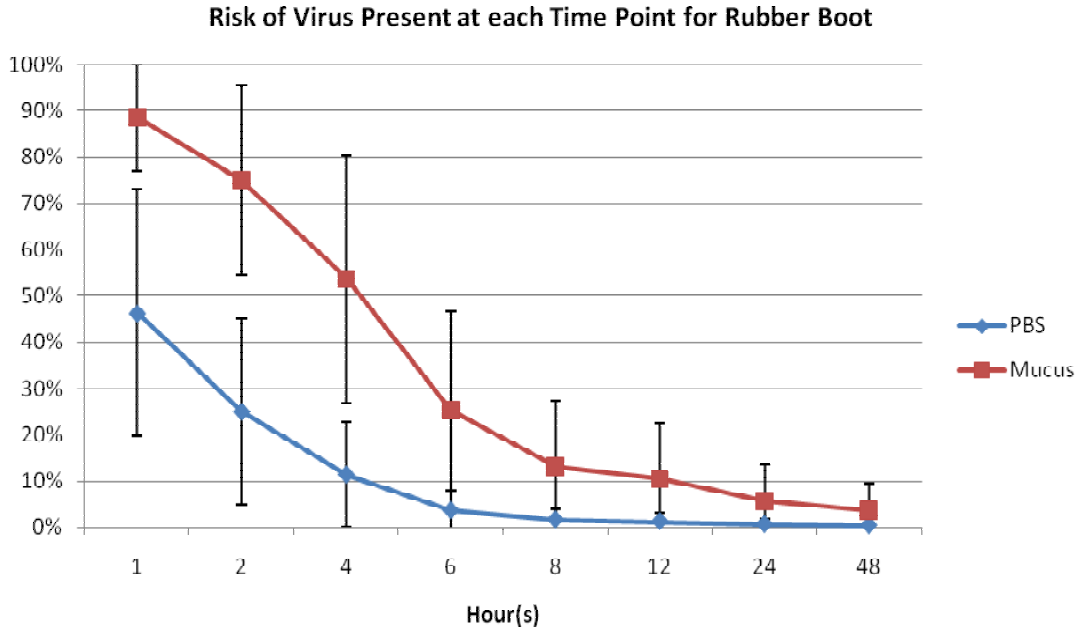| Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Pine	1	PBS	4.77%	-2.05%	11.58%
Pine	2	PBS	1.89%	-1.04%	4.83%
Pine	4	PBS	0.74%	-0.49%	1.96%
Pine	6	PBS	0.22%	-0.17%	0.61%
Pine	8	PBS	0.10%	-0.09%	0.28%
Pine	12	PBS	0.07%	-0.07%	0.22%
Pine	24	PBS	0.04%	-0.04%	0.12%
Pine	48	PBS	0.02%	-0.03%	0.08%
Pine	1	Mucus	31.12%	0.78%	61.46%
Pine	2	Mucus	14.83%	-3.61%	33.26%
Pine	4	Mucus	6.30%	-2.59%	15.19%
Pine	6	Mucus	1.92%	-1.08%	4.92%
Pine	8	Mucus	0.86%	-0.58%	2.30%
Pine	12	Mucus	0.67%	-0.48%	1.81%
Pine	24	Mucus	0.35%	-0.30%	0.99%
Pine	48	Mucus	0.22%	-0.22%	0.65%

Figure 4.6. Model adjusted risk estimates of virus being present on galvanized metal. Based on

three replicates at each time point for each treatment.
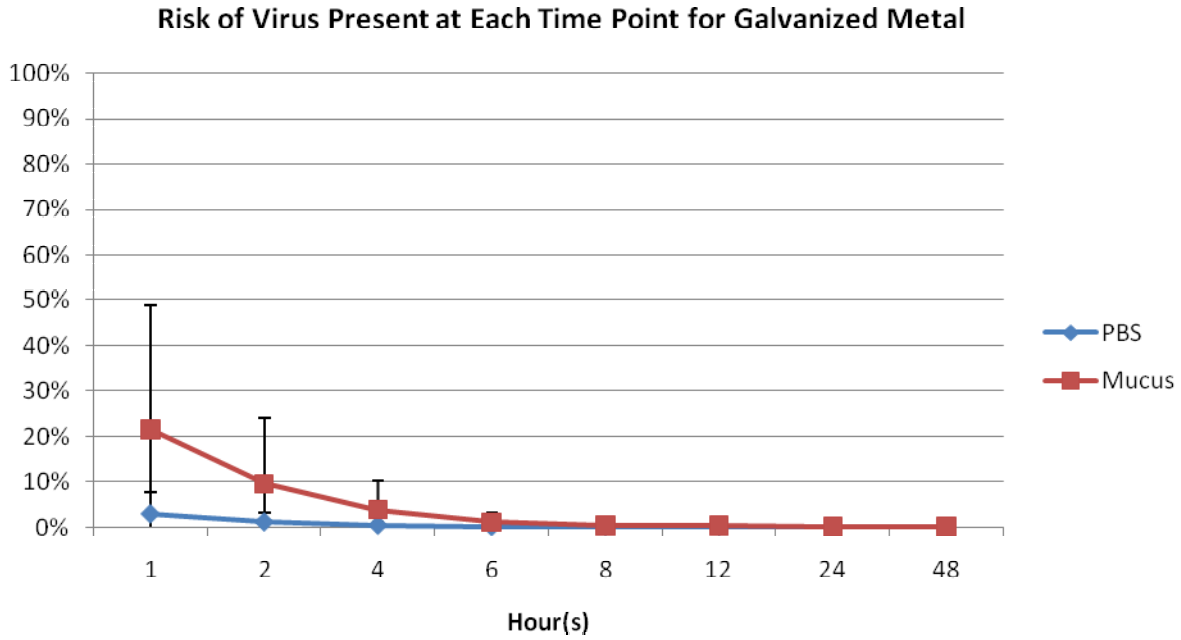
Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Galvanized	1	PBS	2.95%	-1.92%	7.81%
Galvanized	2	PBS	1.16%	-0.88%	3.19%
Galvanized	4	PBS	0.45%	-0.38%	1.28%
Galvanized	6	PBS	0.13%	-0.13%	0.39%
Galvanized	8	PBS	0.06%	-0.06%	0.18%
Galvanized	12	PBS	0.05%	-0.05%	0.14%
Galvanized	24	PBS	0.02%	-0.03%	0.08%
Galvanized	48	PBS	0.01%	-0.02%	0.05%
Galvanized	1	Mucus	21.50%	-5.79%	48.78%
Galvanized	2	Mucus	9.55%	-4.84%	23.94%
Galvanized	4	Mucus	3.92%	-2.51%	10.34%
Galvanized	6	Mucus	1.18%	-0.90%	3.25%
Galvanized	8	Mucus	0.52%	-0.45%	1.50%
Galvanized	12	Mucus	0.41%	-0.36%	1.17%
Galvanized	24	Mucus	0.21%	-0.22%	0.64%
Galvanized	48	Mucus	0.13%	-0.16%	0.42%

Figure 4.7. Model adjusted risk estimates of virus being present on enameled metal. Based on three replicates at each time point for each treatment.


Risk of Virus Present at each Time Point for Enameled Metal
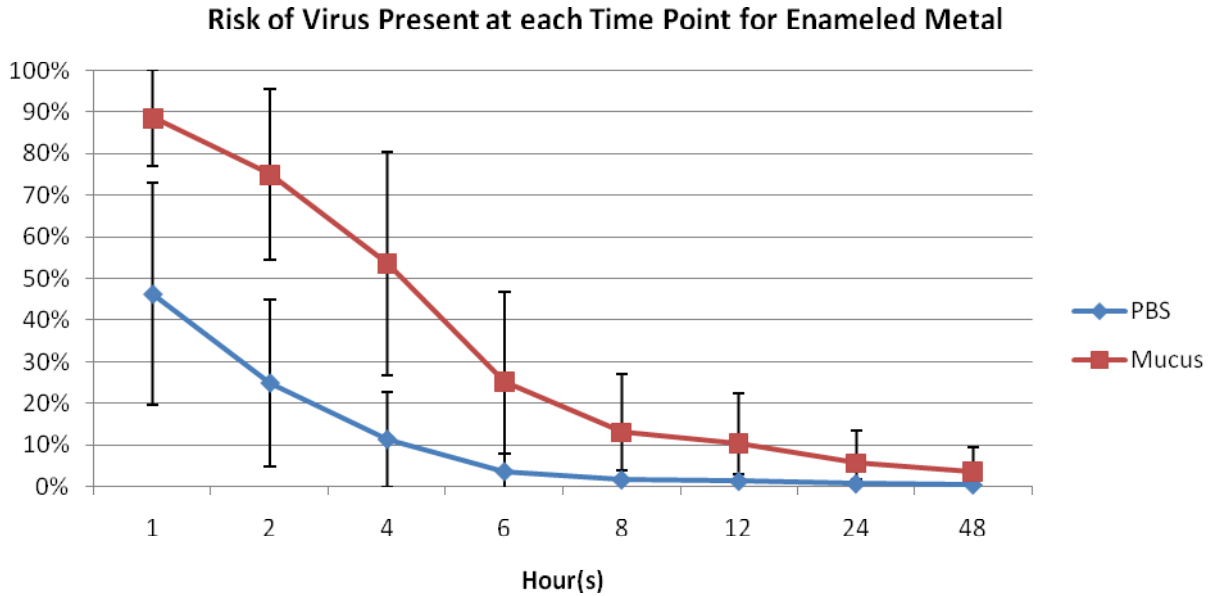
Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Enameled	1	PBS	46.28%	19.54%	73.02%
Enameled	2	PBS	24.93%	4.86%	44.99%
Enameled	4	PBS	11.36%	0.04%	22.69%
Enameled	6	PBS	3.61%	-0.79%	8.01%
Enameled	8	PBS	1.63%	-0.63%	3.88%
Enameled	12	PBS	1.26%	-0.56%	3.09%
Enameled	24	PBS	0.66%	-0.42%	1.74%
Enameled	48	PBS	0.41%	-0.34%	1.16%
Enameled	1	Mucus	88.60%	77.01%	100.18%
Enameled	2	Mucus	74.97%	54.44%	95.50%
Enameled	4	Mucus	53.63%	26.90%	80.35%
Enameled	6	Mucus	25.24%	3.78%	46.70%
Enameled	8	Mucus	12.99%	-1.20%	27.18%
Enameled	12	Mucus	10.35%	-1.80%	22.50%
Enameled	24	Mucus	5.64%	-2.35%	13.63%
Enameled	48	Mucus	3.57%	-2.30%	9.45%

Figure 4.8. Model adjusted risk estimates of virus being present on soil. Based on three replicates at each time point for each treatment.


Risk of Virus Present at each Time Point for Soil
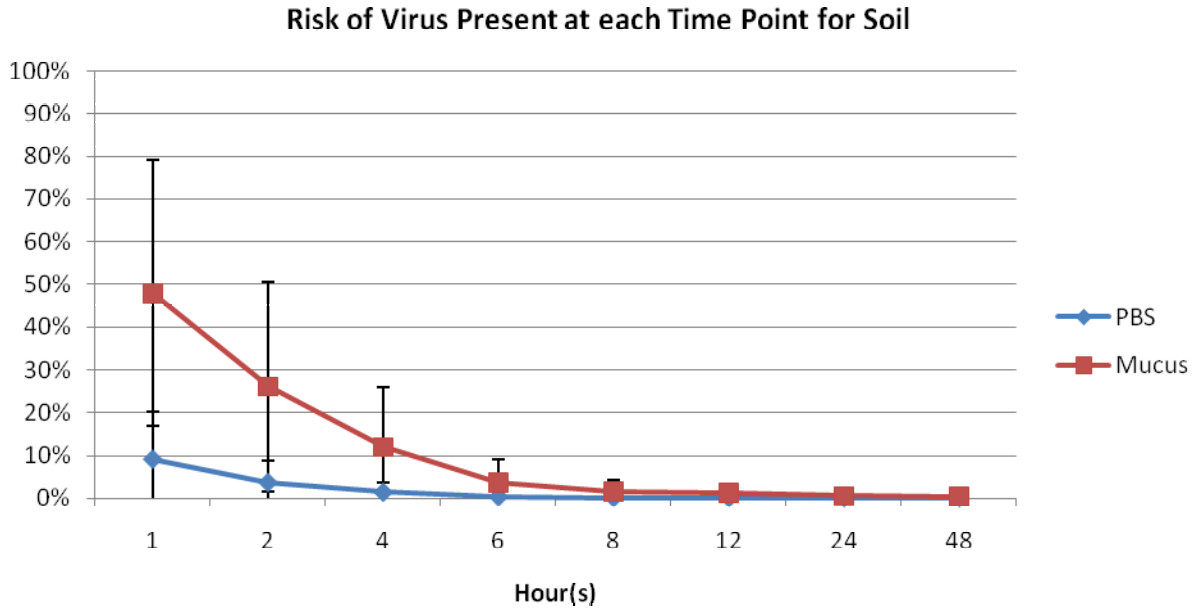
Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Soil	1	PBS	9.24%	-1.80%	20.28%
Soil	2	PBS	3.78%	-1.23%	8.79%
Soil	4	PBS	1.49%	-0.68%	3.66%
Soil	6	PBS	0.44%	-0.27%	1.15%
Soil	8	PBS	0.20%	-0.14%	0.53%
Soil	12	PBS	0.15%	-0.12%	0.42%
Soil	24	PBS	0.08%	-0.07%	0.23%
Soil	48	PBS	0.05%	-0.05%	0.15%
Soil	1	Mucus	47.88%	16.80%	78.97%
Soil	2	Mucus	26.15%	1.65%	50.64%
Soil	4	Mucus	12.03%	-1.88%	25.94%
Soil	6	Mucus	3.84%	-1.36%	9.03%
Soil	8	Mucus	1.73%	-0.85%	4.31%
Soil	12	Mucus	1.35%	-0.73%	3.42%
Soil	24	Mucus	0.70%	-0.50%	1.90%
Soil	48	Mucus	0.44%	-0.39%	1.26%

Figure 4.9. Model adjusted risk estimates of virus being present in PBS. Based on three replicates at each time point for each treatment.

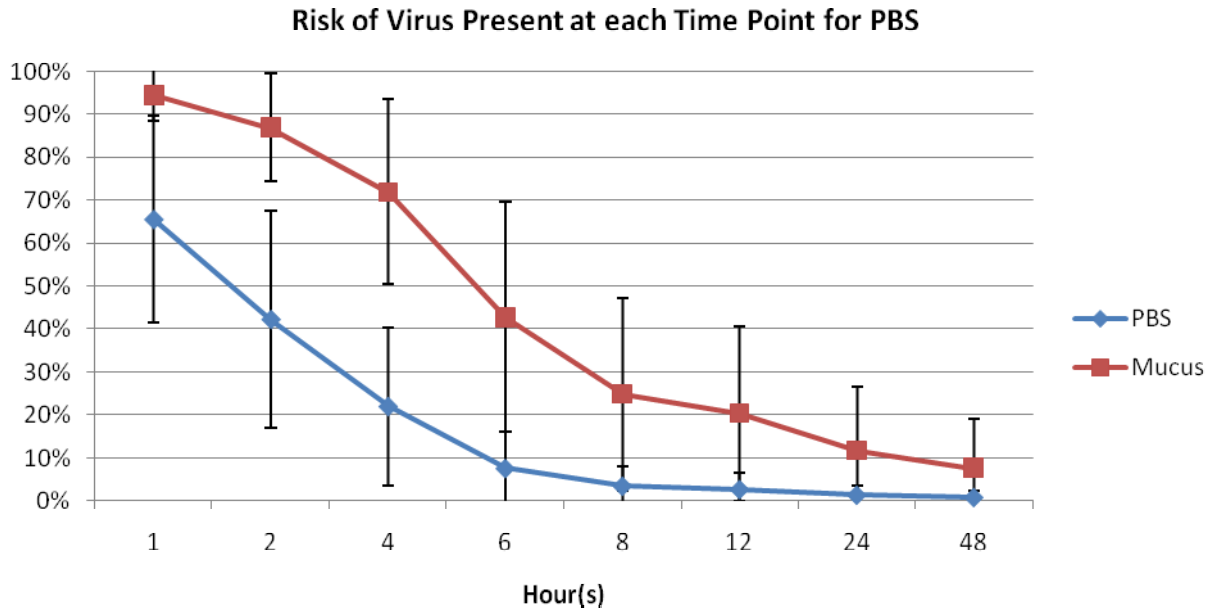**Risk of Virus Present at each Time Point for PBS**

Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
PBS	1	PBS	65.57%	41.58%	89.55%
PBS	2	PBS	42.33%	17.04%	67.62%
PBS	4	PBS	22.08%	3.69%	40.48%
PBS	6	PBS	7.64%	-0.72%	16.00%
PBS	8	PBS	3.53%	-0.97%	8.03%
PBS	12	PBS	2.75%	-0.94%	6.44%
PBS	24	PBS	1.44%	-0.79%	3.68%
PBS	48	PBS	0.90%	-0.68%	2.48%
PBS	1	Mucus	94.50%	88.38%	100.62%
PBS	2	Mucus	86.88%	74.28%	99.47%
PBS	4	Mucus	71.88%	50.36%	93.40%
PBS	6	Mucus	42.73%	15.97%	69.49%
PBS	8	Mucus	24.81%	2.41%	47.21%
PBS	12	Mucus	20.33%	0.02%	40.64%
PBS	24	Mucus	11.67%	-3.27%	26.60%
PBS	48	Mucus	7.57%	-4.03%	19.18%

Figure 4.10. Model adjusted risk estimates of virus being present in water. Based on three replicates at each time point for each treatment.
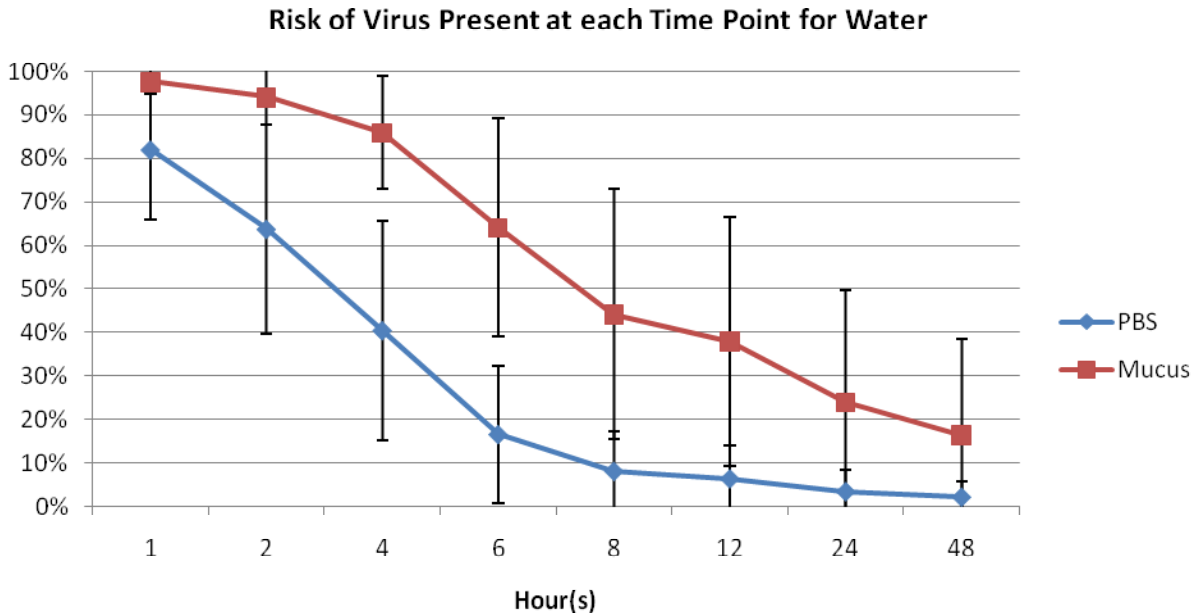

Risk of Virus Present at each Time Point for Water

Fomite	Time (hr)	Treatment	Virus Present	Lower 95% CI	Upper 95% CI
Water	1	PBS	82.02%	66.06%	97.99%
Water	2	PBS	63.75%	39.74%	87.76%
Water	4	PBS	40.44%	15.16%	65.72%
Water	6	PBS	16.54%	0.80%	32.28%
Water	8	PBS	8.06%	-1.25%	17.37%
Water	12	PBS	6.35%	-1.43%	14.13%
Water	24	PBS	3.39%	-1.51%	8.29%
Water	48	PBS	2.13%	-1.41%	5.67%
Water	1	Mucus	97.63%	94.77%	100.48%
Water	2	Mucus	94.07%	87.67%	100.47%
Water	4	Mucus	85.96%	72.85%	99.08%
Water	6	Mucus	64.13%	39.13%	89.13%
Water	8	Mucus	44.15%	15.36%	72.94%
Water	12	Mucus	37.94%	9.34%	66.53%
Water	24	Mucus	24.04%	-1.51%	49.60%
Water	48	Mucus	16.41%	-5.65%	3.85%

CHAPTER 5 - Characterization of Testing and Longitudinal Prevalences for PI-BVDV Cattle in the Beef Industry

ABSTRACT

The objective of this study was to evaluate the sample submission forms from a commercial PI-BVDV testing laboratory located in central Kansas. In all, 1,490 sample submission forms, representing 153,716 head of cattle, were partially or fully completed. After reviewing the completed data set, we were able to analyze the PI-BVDV prevalence by weight, year, month, and by submitting client (cow-calf, stocker, or feedlot). The overall PI-BVDV prevalence for all submitted samples was 0.34% (524/153,716). Cattle with a weight of 300 lbs. or less had the highest prevalence (0.43%) and cattle with a weight of 701 lbs. or greater had the lowest prevalence (0.23%). The model adjusted odds ratio (OR) of testing PI-BVDV positive for cattle weighing between 301 to $\leq$ 500 lbs., 501 to $\leq$ 700 lbs. or $\geq$ 701 lbs. compared to cattle weighing $\leq$ 300 lbs. was 0.80 (95 % CI 0.58 – 1.06), 0.6 (95 % CI 0.45 – 0.80) and 0.73 (95% CI 0.56 – 0.96), respectively. The highest yearly prevalence (Jan. – Dec.) occurred during 2007 (0.37%). The model adjusted ORs of cattle testing PI-BVDV in 2006 or 2008 compared to cattle being tested in 2007 were 0.99 (95 % CI 0.82 – 1.20) and 0.73 (95 % CI 0.54 – 0.99), respectively. The peak number of head tested in a month occurred during October. For the PI-BVDV prevalence by month, January had the highest prevalence (0.44%), and the month of October had the lowest prevalence (0.21%). Based on model adjusted ORs of cattle testing PI-BVDV in October compared to cattle being tested in any other month, the months that were suggested to have an increase in the point estimate for the risk of indentifying PI-BVDV animals

were January (OR = 2.63; 95 % CI 1.74 – 3.98), February (OR = 2.38; 95 % CI 1.61 – 3.53),

March (OR = 1.9; 95 % CI 1.29 – 2.78), May (OR = 2.53; 95 % CI 1.65 – 3.89), September (OR

= 2.06; 95 % CI 1.33 – 3.2), November (OR = 2.06; 95 % CI 1.46 – 2.90), and December (OR =

2.06; 95 % CI 1.34 – 2.8). When the submitting client was identified as primarily involved in the

cow-calf, stocker, or feedlot industry, the overall prevalence by operation was 0.38%, 0.55%,

and 0.31%, respectively. The model adjusted OR of testing PI-BVDV for cow-calf or stocker

cattle compared to feedlot cattle was 1.32 (95 % CI 0.93 – 1.88) and 2.10 (95 % CI 1.65 – 2.69),

respectively.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an important pathogen of cattle and can have a

diversity of clinical outcomes that are largely dependent on the host and the infecting BVDV

strain.   A detrimental clinical outcome is the manifestation of an *in utero* infection between 30

and 125 days of gestation with a non-cytopathic biotype of BVDV. If a fetus survives the

infection, it can cause a persistently infected BVDV individual (Baker, 1985).   The significance

of the PI-BVDV individual is the continual shedding of BVDV into the environment (Bolin et al.

1987), which can allow for the propagation of the virus through not only acute infections, but

also through the establishment of additional persistently infected individuals. The risk of initial

treatment for bovine respiratory disease (BRD) was 43 % greater in cattle exposed to a PI calf

(Loneragan et al, 2003). Persistently infected calves tend to have lower growth rates and often

die from classic mucosal disease or other diseases during the feeding period (Taylor et al, 1997).

To date, several research groups have evaluated the point prevalence of PI-BVDV

individuals. With respect to beef cattle, the prevalence of PI-BVDV animals entering

conventional feeding systems has ranged from 0.17 - 0.4 % (Loneragan et al, 2003, Larson et al, 2005, Stevens et al, 2007, O'Connor et al, 2005). Furthermore, a recent study in Iowa evaluated the prevalence of PI-BVDV animals in spring born beef calves (n = 12,030) using antigen capture ELISA (ACE) and immunohistochemistry (IHC) to detect positive animals (O'Connor et al, 2008). In that study, calves were first tested by ACE (prevalence = 0.20%) and later tested by IHC (prevalence = 0.09%). Furthermore, the herd level prevalence (n = 102), with at least one positive PI-BVDV calf, was reported to be 10.8 % by ACE and 3.9 % by IHC (O'Connor et al, 2008). Interestingly, at the herd level, in a 5 state US based study, 3 % of randomly selected cow herds contained PI cattle (Wittum et al, 2001).

Montana State University's Beef Quality Assurance program, in association with the Montana Stockgrower's Association, has developed the Montana BVD-PI Herd Biosecurity Project. The aims of the project are to reduce disease potential in individual cow herds, add value to commercial and seedstock cattle, and be a template for on-ranch biosecurity. The program seeks to screen the whole herd before breeding to avoid exposing pregnant cows to the BVDV during early gestation and establish surveillance by screening aborted fetuses, stillborns and pre-weaning deaths. Initial screening is performed using pooled reverse transcriptase polymerase chain reaction (RT-PCR). If the RT-PCR is positive, then 3 to 4 weeks post initial testing ear notch samples are collected from all suspect animals for confirmatory testing by immunohistochemistry (IHC). In 2007, 408 herds were screened and 31 herds contained at least 1 PI animal (7.8%). The 408 herds represented approximately 106,600 head and there were 110 confirmed PI animals (0.13 % or 1.03 PIs/1000 head).

In 2002, the Academy of Veterinary Consultants (AVC) presented a position statement on BVDV for eventual eradication of the virus in North America (Grotelueschen, 2002). The BVDV position statement reads:

> "The beef and dairy industries suffer enormous loss due to the effects of bovine viral diarrhea virus (BVDV) infection. The highly mutable nature of the BVDV and the emergence of highly virulent strains of BVDV contribute to limited success of present control programs. Also persistently infected cattle are the primary source of infection and effective testing procedures are available to identify those infected carriers. Therefore, it is the resolve of the Academy of Veterinary Consultants the beef and dairy industries adopt measures to control and target eventual eradication of BVDV from North America."

<div align="right">Academy of Veterinary Consultants – November, 2001</div>

The AVC's position statement was endorsed in September of 2002 by the American Association of Bovine Practitioner's Board of Directors and endorsed again in February of 2003 by the National Cattleman's Beef Association's Animal Health and Well Being Committee.

If BVDV is to be eradicated, it must first be controlled. To control BVDV, transmission must be prevented, the agent must be eliminated, and immunity must be increased. Biosecurity and biocontainment are important considerations for establishing control and the subsequent maintenance of eradication. The fundamental components of BVDV control plan are: 1) prevent BVDV entry into herds; 2) vaccination in the event exposure occurs; 3) indentify BVDV infected herds and eliminate PI animals; and 4) monitor and document success (Smith et al, 2004).

Current issues that are being addressed to support eradication efforts are the development of simplified biosecurity and biocontainment formats, development of herd and individual animal level categories, and validate and determine the most appropriate use(s) of current and future tests for diagnosis of PI-BVDV status (Maas, 2007).

With respect to the need for validation and determination of the most appropriate use(s) of current and future tests for the diagnosis of PI-BVDV status, there are several methods for detecting PI-BVDV individuals: immunohistochemistry (IHC) on ear notch biopsies, microplate virus isolation, antigen capture enzyme linked immunosorbent assay (ACE) for serum and fresh tissue, polymerase chain reaction (PCR), and virus isolation. Although there are a number of tests available to practitioners and producers, the BVDV antigen capture enzyme linked immunosorbent assay (ACE) is often used for the initial screening of feedlot cattle. A number of sample handling practices have been evaluated and found to have little impact on test sensitivity and specificity of ACE testing for BVDV (Funk et al, 2008; Reed et al, 2008). Recently, several tests for BVDV were applied to samples collected monthly for an 11 month period from 12 PI BVDV animals (Fulton et al, 2009). The test population was comprised of the genotypes and subgenotypes of BVDV 1a, 1b, and 2a. In that study, the antigen capture ELISA (ACE) and IHC tests on ear notches had acceptable levels of agreements between the two tests throughout the study period. Therefore, with regards to our ability to accurately identify PI-BVDV animals as the first step in an eradication program, the use of ACE testing may be a useful tool for the identification and the removal of PI-BVDV animals.

To date, there have been no longitudinal studies to evaluate the prevalence of PI-BVDV individuals. Therefore, the objectives of this study were to characterize the PI-BVDV prevalence by weight, month, year, and by submitting client (cow-calf, stocker, or feedlot).

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MATERIALS AND METHODS

Sample Population

A standardized form was used for gathering data for ear notch biopsy samples being submitted for ACE testing at a commercial testing facility. Data was obtained from sample submission forms from January 2006 until April 2008. Data obtained consisted of yard, date, pen, lot, tag numbers, total number of vials, tag color, average weight, order buyer, and state of origin. Additionally, for further characterization of the test animal(s), the client was asked to identify the origin of the cattle as sale barn origin, started, yearling, Mexican, and/or natural. The owner of the testing facility, who was also the consulting veterinarian, identified each client who submitted samples for testing as a cow-calf, stocker, or feedlot operator.

Antigen Capture ELISA

Detection of BVDV antigen in skin specimens (ear notch) was performed using a commercial ACE kit.[i] Results were calculated by the following equation: standardized optical density (OD) = (raw OD of sample – raw OD of negative control)/(raw OD of positive control – raw OD of negative control). Samples with standardized OD values < 0.20 were considered negative, and those with OD values > 0.39 were considered positive. Samples with values from 0.2 to 0.39 were retested with detector reagents with or without antibody. Upon secondary analysis, no animals had values from 0.20 – 0.39.

Data Analysis and Model Building

Data were then imported from the spreadsheets into Stata 10 (Statacorp; College Station, TX) and reformatted as necessary. The data were reported on the aggregate level such that the

authors only knew the number tested and the number positive for each lot of cattle. The data were then expanded using lot as the cluster identifier to create a dataset that contained an individual observation for each animal tested. Several new potential predictor variables were created from the data to be used in the model building process. Weight was transformed into a four category variable ($\leq$ 300 lbs., 301 - 500 lbs., 501 - 700 lbs., $\geq$700 lbs.) to better communicate perceived risks in these weights of cattle. Variables were then analyzed for an association with a positive BVDV-PI test result using a Pearson's chi-square test. Those with an association were entered as fixed effects into the predictive and descriptive models.

The predictive and descriptive models were built using manual techniques using forward selection. All variables associated ($P < 0.05$) with the outcome of interest in the univariable analysis were included in the main effects models. All variables and their interactions were assessed using likelihood ratio tests or Wald-type tests where appropriate. For the predictive model, only variables that were found to be statistically significant ($P < 0.05$) were retained in the model. For the descriptive model, all variables of interest were retained in the model regardless of significance. All referent variable categories, with the exception of weight category, were manually set to the category in which the largest number of observations occurred in order to make the model more robust.

As variables were added to the model all variables were examined for evidence of confounding. A confounder was defined as any non-intervening variable that resulted in a 20% or greater change in the coefficient of a significant variable when added to the model (Dohoo et al, 2003). If two variables were identified as confounders, only one variable was retained in the model.

RESULTS

In all, 1,490 sample submission forms, representing 153,716 head of cattle, were partially or fully completed. Five hundred and twenty four (524) head of the 153,716 head tested positive by ACE testing, which was an overall prevalence of 0.34 %. The mean number of head tested per sample submission form was 112 with a median of 91. For submission forms that included the weight of the animals and the date of testing, 379 of the 118,084 animals tested positive by ACE for BVDV, which was an overall prevalence of 0.32 % (Table 5.1and Figure 5.1). Longitudinally, the range of the average weight of cattle that tested positive was 344 – 695 lbs (Figure 5.2). Interestingly, the longitudinal range of the average weight of cattle that tested negative was 345 – 744 lbs (Figure 5.2). Further characterization of weight revealed that calves with a weight of 300 lbs. or less had the highest prevalence (0.43 %; Figure 5.3), whereas calves with a weight of 701 lbs. or greater had the lowest prevalence (0.23 %; Figure 5.3).

For the predictive model, all factors examined were not significant in the prediction of having a positive PI-BVDV animal. Therefore, a descriptive model was developed to demonstrate the differences in factors of interest. The final model included weight, year, month, and submitting client categorical variables. The model adjusted odds ratio (OR) of testing PI-BVDV positive for cattle weighing between 301 to ≤ 500 lbs. compared to cattle weighing ≤ 300 lbs. was 0.80 (95 % CI, 0.58 – 1.06), which suggested a point estimate of a 20 % decrease in the risk of identifying PI-BVDV positive cattle weighing between 301 to $\leq$ 500 lbs (Table 5.2). Additionally, the model adjusted ORs of testing PI-BVDV positive of cattle weighing between 501 to ≤ 700 lbs or $x \geq 701$ compared to cattle weighing ≤ 300 lbs were 0.6 (95 % CI 0.45 – 0.80) and 0.73 (95% CI 0.56 – 0.96), respectively, which suggested a point estimate of a 40 % and 27 % decrease in the risk of identifying PI-BVDV positive cattle, respectively.

We also characterized the prevalence of PI-BVDV prevalence by year of testing. Of the three years that data was collected, the highest yearly prevalence occurred during 2007 (0.37 %; Table 5.3 and Figure 5.1). The model adjusted ORs of cattle testing PI-BVDV in 2006 or 2008 compared to cattle being tested in 2007 was 0.99 (95 % CI 0.82 – 1.20) and 0.73 (95 % CI 0.54 – 0.99), respectively (Table 5.4). In both 2006 and 2007, the peak number of head tested in a month occurred during October (Table 5.5; Figure 5.4). The month of January had the highest prevalence (0.44 %; Table 5.5; Figure 5.5), while the month of October had the lowest prevalence (0.21 %; Table 5.5). The model adjusted ORs of cattle testing PI-BVDV in October compared to cattle being tested in other months and being PI-BVDV positive, were completed and the data is summarized in Table 5.6. The model adjusted ORs for January (OR = 2.63; 95 % CI 1.74 – 3.98), February (OR = 2.38; 95 % CI 1.61 – 3.53), March (OR = 1.9; 95 % CI 1.29 – 2.78), May (OR = 2.53; 95 % CI 1.65 – 3.89), September (OR = 2.06; 95 % CI 1.33 – 3.2), November (OR = 2.06; 95 % CI 1.46 – 2.90), and December (OR = 2.06; 95 % CI 1.34 – 2.8) suggested these months have an increase in the point estimate compared to October for the risk of indentifying PI- BVDV animals.

We characterized the prevalence by the submitting client. There were three main categories for submitting clients: cow-calf, stocker, or feedlot. For clients that were identified as primarily involved in the cow-calf industry, there were 209 lots, which represented 11,116 head (Table 5.6). There were 16 lots that contained at least 1 PI animal (7.6 %; 16/209). There were a total of 42 positive animals, which represented an overall prevalence of 0.38%. The average and median for the cattle that were tested per submission were 53 and 33, respectively. The peak prevalence of PI animals occurred in November 2006 (2.7 %; Figure 5.6). During November

2006, 769 head were tested and 21 PI animals were identified. The peak number of animals tested occurred in October 2006 (n = 1088).

For clients that were identified as primarily involved in the stocker industry, there were 198 lots, which represented 16,086 head (Table 5.6). There were 43 lots that contained at least 1 PI-BVDV animal (21.7 %; 43/198). There were a total of 89 positive animals, which represented an overall prevalence of 0.55%. The average and median for the cattle that were tested per submission were 81 and 79, respectively. The peak prevalence of PI animals occurred in January 2008 (4.0%; Figure 5.7).   During January 2008, 4 lots of cattle were tested, which represented a total of 174 head. Seven of the 174 head tested in January 2008 were positive by ACE. Interestingly, during January 2008, one of the lots tested contained 9 head that were purchased from a sale barn and contained 4 calves that were PI-BVDV. The peak number of head tested occurred in October 2006 (n = 3,198).

For clients that were identified as primarily involved in the feedlot industry, there were 1,067 lots, which represented 125,542 head (Table 5.6). There were 246 lots that contained at least 1 PI-BVDV animal (23.1%; 246/1,067). Additionally, there were 391 positive animals, which represented an overall prevalence of 0.31%. The average and median for the cattle that were tested per submission were 117 and 92, respectively.   The peak prevalence of PI animals occurred in May 2007 (0.8%; Figure 5.8).   During May 2007, 2,249 head were tested and 18 tested positive by ACE.   The number of head tested peaked during October of 2006 (n = 11,728). The model adjusted OR of testing PI-BVDV for cow-calf or stocker cattle compared to feedlot cattle was 1.32 (95 % CI 0.93 – 1.88) and 2.10 (95 % CI 1.65 – 2.69), respectively (Table 5.7).

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DISCUSSION

The objective of this study was to evaluate the sample submission forms from a commercial BVDV testing laboratory located in central Kansas. Sample submission forms were collected that represented a continuous 29 month testing period (Jan. 2006 – Apr. 2008). The sample submission form that clients were asked to complete at the time of specimen submission include an area for the client to provide information about the sample population. Specifically, feedlot (if applicable), date, pen number, lot numbers, tag numbers, total number of vials, tag color, average weight, order buyer, and state of origin. In all, 1,490 submission forms were used to complete the data set. The majority of sample submission forms were only partially completed. After reviewing the completed data set, we were able to analyze the prevalence by weight, month, year, and by submitting client (cow-calf, stocker, or feedlot).

The overall prevalence of 0.34 % is consistent with other published studies (Loneragan et al, 2003, Larson et al, 2005, Stevens et al, 2007, O'Connor et al, 2005). The overall prevalences for 2006, 2007, and 2008 were 0.32 %, 0.37%, and 0.31 %, respectively. The ORs of cattle being tested in 2007 and PI-BVDV positive, compared to cattle being tested in 2006 or 2008 and PI-BVDV positive, was 0.99 (95 % CI 0.82 – 1.20) and 0.73 (95 % CI 0.54 – 0.99), respectively. The OR for 2006 suggested that the likelihood of identifying PI-BVDV cattle in 2006 (P = 0.94) was not different than in 2007. However, the OR for 2008 suggested a point estimate decrease of 27 % in identifying PI-BVDV cattle in 2008 (P = 0.046). The exact reason for the decrease in 2008 is not known, but may be attributable to only having the first 5 months of data from 2008, cattle arriving from different sources, increased number of vaccinated cattle or other reasons related to increased industry awareness of PI-BVDV contributing factors.

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We were able to characterize the frequency of testing by month and prevalence by month. Regardless of year or operation, the peak number of head tested in any month occurred during October. The increased number of head tested was most likely associated with the seasonal placement of cattle into the feedlot and subsequent testing of spring born calves. The month of May had the highest prevalence. The exact reason for this is not known; however, in months when fewer cattle were tested, the prevalence was increased. One reason for the increased prevalence, while the number of head tested was decreased, could be that the tested animals met the criteria for diagnostic testing because of health status, case definition, or biosecurity concerns. The model adjusted ORs of testing PI-BVDV positive for cattle being tested in October compared to cattle being tested in any other month suggested that there was an increase in the point estimate for the risk of identifying PI-BVDV animals in January, February, March, May, September, November, and December.

Characterization of prevalence by weight was evaluated. Cattle were categorized into one of four weight classes: $x \leq 300$, $301 \leq 500$, $501 \leq 700$, or $x \geq 701$. The corresponding prevalences were 0.43 %, 0.37 %, 0.27 %, and 0.23 %, respectively. The model adjusted odds ratio of testing BVDV-PI positive for cattle weighing between 301 to ≤ 500 lbs. compared to cattle weighing ≤ 300 lbs. was 0.80 (95 % CI, 0.58 – 1.06). This suggests a point estimate of a 20 % decrease in the risk of identifying PI-BVDV positive cattle weighing between 301 to ≤ 500 lbs. Additionally, the ORs of cattle weighing between 501 to ≤ 700 lbs or ≥ 701 compared to cattle weighing ≤ 300 lbs were 0.6 (95 % CI 0.45 – 0.80) and 0.73 (95% CI 0.56 – 0.96), respectively, which suggested a point estimate of a 40 % and 27 % decrease in the risk of identifying PI-BVDV positive cattle, respectively. Therefore, the lightest weight cattle, $x \leq 300$, were at highest risk for identifying positive animals in this dataset.

We were able to categorize the submitting client as either being primarily involved in cow-calf, stocker, or feedlot operations. For clients that were identified as primarily involved in the cow-calf, stocker, or feedlot industry, the overall prevalence by operation was 0.38%, 0.55%, and 0.31%, respectively. Further characterization of cow-calf operators revealed that 88 operators had submitted samples. Of the 88 operators, 7 had at least 1 PI-BVDV positive animals (7.9 %; 7/88). This finding is strikingly similar to that of the Montana BVD-PI Herd Biosecurity Project in 2007, where 408 herds were screened and 31 herds contained at least 1 PI animal (7.8%). The 408 herds represented approximately 106,600 head and 110 PI animals were positive (0.13 % or 1.03 PIs/1000 head). Although at the herd level, our results were similar, there was a divergence when the total number of head tested was evaluated. In our analysis, the 88 herds represented 11,116 head and 42 PI animals were positive (0.38 % or 3.8 PIs/1000 head). The exact reason for the increased prevalence in the central KS herds is not known. However, the difference for the individual animal prevalence may be attributable to the willingness of progressive Montana cow-calf producers to participate in a program associated with a beef quality assurance program. The willingness to participate in such a program might infer a progressive management style with the expectation that few calves would be identified as PI. Whereas in the central KS herds, the willingness to participate was not mandated, nor subsidized, and may have been for personal gain to assist in marketing, maintenance or implementation of biosecurity, or meeting the criteria of a case definition which demonstrated a need for herd testing.

For operators that were identified as being involved in the stocker industry, the prevalence was the highest of the three potential operations (0.55 %; 88/16,085). The reason for the increased prevalence is most likely associated with increased concentration of cattle from

potentially diverse health backgrounds. Given that the average cow herd in the United States is approximately 30 head, and the average number of head tested by stocker operators was 79, it seems likely that previous management is an important consideration for the increased prevalence. Additionally, the act of purchasing calves with diverse health backgrounds may have prompted increased testing, and through increased testing the prevalence may be the true prevalence rather than a prevalence derived from more strategic and volunteered samples. Interestingly, the prevalence for feedlot operators was 0.31%. This is an impressive reduction from the stocker prevalence of 0.55% that may be attributed to increase culling of BVDV-PI animals from backgrounding or stocker phases of the production system or an increased death rate. It has been reported that PI-BVDV calves are often susceptible to mucosal disease. Mucosal disease, which typically is assumed to be 100% fatal, occurs when a cytopathic BVDV with a similar antigenic structure to the non-cytopathic strain that created the PI-BVDV calf avoids immune recognition. By avoiding the immune system, the virus ravages the calf, which culminates in death. If the true prevalence is 0.55% for stocker operations, it would be important to characterize mortality of stocker calves to determine if there was an increase in mortality associated with PI-BVDV animals.

Lastly, the model adjusted odds ratio (OR) of testing BVDV-PI positive of cow-calf or stocker cattle compared to feedlot cattle, was 1.32 (95 % CI 0.93 – 1.88) and 2.10 (95 % CI 1.65 – 2.69), respectively. The OR for cow-calf suggests there is a tendency for a point estimate increase of 32 % in identifying PI-BVDV cattle in cow-calf operations (P = 0.11). The OR for stocker suggests that there is a point estimate increase of 210 % in identifying PI-BVDV in stocker operations. This data taken together strongly implies that if BVDV is to be eradicated, testing and removal programs must be initiated at the level of the cow-calf producer. In our

current study, there were more feedlot animals tested than animals from cow-calf operations. Even more importantly, we test animals at the terminal point of the industry, when there is sufficient evidence to support that PI-BVDV individuals have little, if any, effect on the performance, health, and carcass characteristics of feedlot pen mates (Booker et al, 2008, Elam et al, 2008, Stevens et al, 2007 and 2009). However, there is at least one published report showing a negative impact of PI-BVDV animals in a starter feedlot (Hessman et al, 2009). The authors in that study found that exposure of the general population of feedlot cattle to PI-BVDV animals resulted in substantial costs attributable to negative effects on performance and increased fatalities. The reason why marketing of PI-BVDV testing is so effective at the feedlot level, with the exception of the time of birth and at branding, is because it is a time in the production system that allows for easy sampling and compliance for removal of positive animals.

How can we be successful in eradication of PI-BVDV at the cow-calf level? It seems unlikely that we can pick a month for testing that would give an edge over any other month. The best time for sampling would be at birth and removal of positive animals as soon as possible. By testing at birth and removing soon thereafter, potentially the cycle of transmission for the herd would be broken. However, the veterinarians, beef extension coordinators, and other pivotal industry leaders need to champion the cause for testing. If we test and remove positive individuals, institute appropriate vaccination programs, and implement biosecurity plans, we will have a firm foundation for eradication. There is one other very important concern for success based on a study conducted by O'Connor et al (2007). While trying to determine the prevalence of PI-BVDV beef cow-calf herds through a voluntary screening project, one very important pitfall was identified. Specifically, of the 131 recruited producers, only 102 actually submitted samples, nearly a quarter dropped out because of an inconvenience to collect ear notch samples.

Furthermore, many of the producers in the study did not submit the samples as requested. The authors requested that each producer ship his or her samples in coolers with ice packs; however, more often than not, many samples arrived in boxes with no insulation or cold packs. Also, to save money on shipping many producers stored samples until calving was complete. Based on this, it seems that if testing is to be initiated, it would be best to test and remove prior to breeding to break the transmission cycle.

An eradication program for BVDV is possible, but the logistics for success need to be carefully evaluated, not only for compliance at the level of testing, but to ensure proper specimen management. Additionally, veterinarians, beef extension coordinators, and other key opinion leaders need to be involved to initiate, maintain, and complete the project. An ideal eradication program would be initiated at the cow-calf level with testing prior to breeding and utilize the ACE for determining test positive and negative animals.

CONCLUSIONS

Cattle that weighed less than 300 lbs. had a greater likelihood of being PI-positive than cattle with increased weights. Several months of the year had a greater likelihood of having PI-positive animals. Based on operation, cow-calf and stocker operations had a greater likelihood of having PI-positive animals as compared to feedlots.

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Table 5.1. The weight class tested and the number of lots, calves, and PI-BVDV positive calves with corresponding prevalence.

Weight (lbs.)	# of Lots	# of Calves	# of PI – Positive	Raw Prevalence %
≤ 300	149	20,640	89	0.43
301 ≤ 500	240	29,971	112	0.37
501 ≤ 700	502	52,476	144	0.27
≥ 701	166	14,997	34	0.23
Total	1,057	118,084	379	0.32

Table 5.2 Association between weight and PI-BVDV prevalence.

Weight of Tested Cattle (lbs.)	OR	95 % CI	P value
x ≤ 300	1.00	NA	NA
301 ≤ 500	0.80	0.60 – 1.07	0.128
501 ≤ 700	0.60	0.46 – 0.80	0.000
701 ≤ x	0.73	0.56 – 0.96	0.023

Table 5.3   Characterization of PI-BVDV Prevalence by year of testing.

Year	# of Lots	# of Calves	# of PI – Positive	Raw Prevalence %
2006	654	66,174	215	0.32
2007	599	64,833	240	0.37
2008	227	22,709	67	0.31
Total	1,480	152,632	522	0.34

Table 5.4. Association between year and PI-BVDV prevalence.

Year Tested	OR	95 % CI	P value
2006	0.99	0.82 – 1.20	0.94
2007	1.00	NA	NA
2008	0.73	0.54 – 0.99	0.046

Table 5.5. Prevalence by month of testing. The total number of head tested each month was summed and prevalence calculated.

Month Tested	# of Lots	# Head Tested	# Head Positive	Raw Prevalence %
January	107	11,105	49	0.44
February	158	16,294	63	0.39
March	181	16,524	59	0.36
April	127	9,810	26	0.27
May	78	7,327	38	0.52
June	52	5,771	12	0.21
July	65	6,507	24	0.37
August	53	6,466	16	0.25
September	88	8,652	34	0.39
October	227	26,783	56	0.21
November	190	20,455	80	0.39
December	154	16,398	65	0.40
Total	1,480	152,092	522	0.34

Table 5.6. Association between month of testing and PI-BVDV prevalence.

Month Tested	OR	95 % CI	P-Value
January	2.63	1.75 – 3.98	0.00
February	2.38	1.60 – 3.53	0.00
March	1.90	1.29 – 2.78	0.001
April	1.38	0.85 – 2.24	0.20
May	2.53	1.64 – 3.89	0.00
June	1.02	0.54 – 1.93	0.94
July	1.82	0.12 – 2.98	0.016
August	1.31	0.74 – 2.34	0.352
September	2.06	1.33 – 3.2	0.001
October	1.00	NA	NA
November	2.06	1.34 – 2.79	0.000
December	1.94	1.34 – 2.8	0.000

Table 5.7. Prevalence based on operation

Operation	# of Lots	# of Calves	# of PI – Positive	Raw Prevalence %
Cow-Calf	209	11,116	42	0.38
Stocker	198	16,085	88	0.55
Feedlot	1,067	125,542	391	0.31
Total	1,474	152,743	521	0.34

Table 5.8. Association between operation and PI-BVDV prevalence.

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Operation	OR	95 % CI	P value
Cow-Calf	1.33	0.94 – 1.88	0.11
Stocker	2.11	1.65 – 2.69	0.00
Feedlot	1.00	NA	NA

**Figure 5.1.** The longitudinal prevalence of all cattle tested regardless of weight.

Overall: Number of Head Tested vs. Prevalence
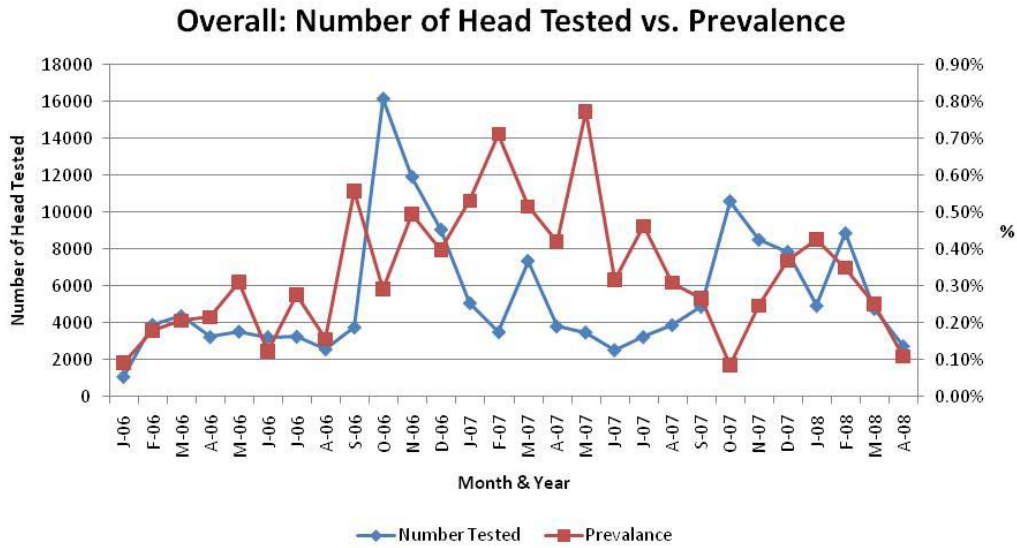
Figure 5.2. The longitudinal average weight of cattle that tested PI-BVDV negative or positive.

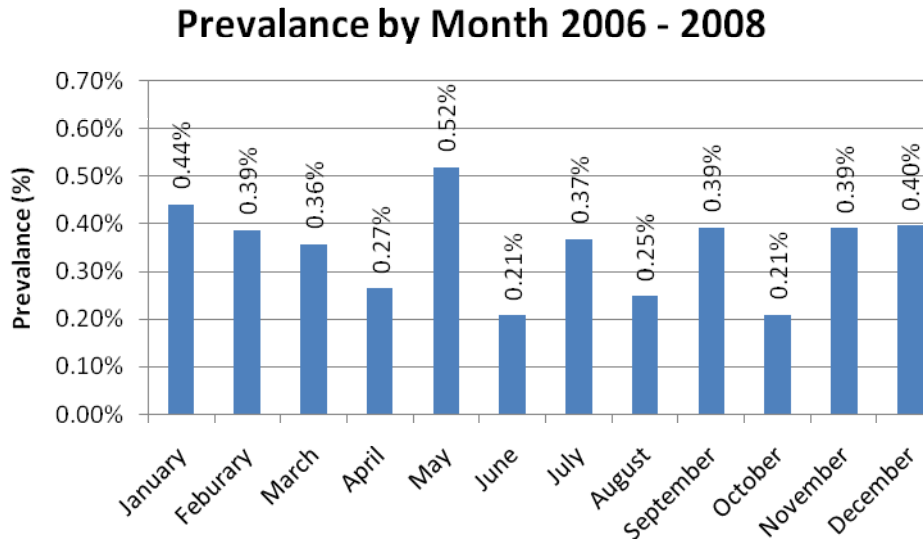Average Weight of Test (-) vs. Average Weight of Test (+)
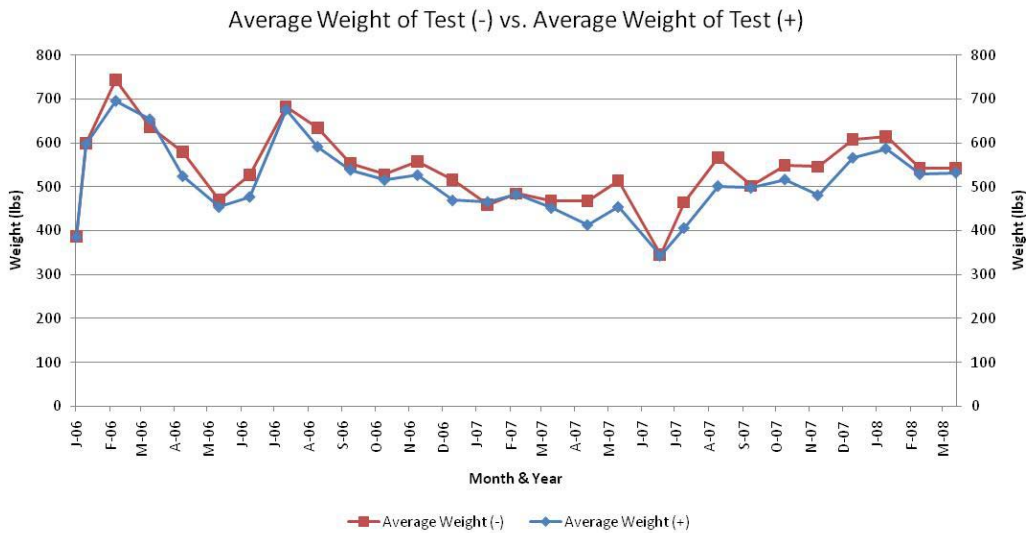
Figure 5.3. Prevalence of animals that tested postive by ACE by weight class.
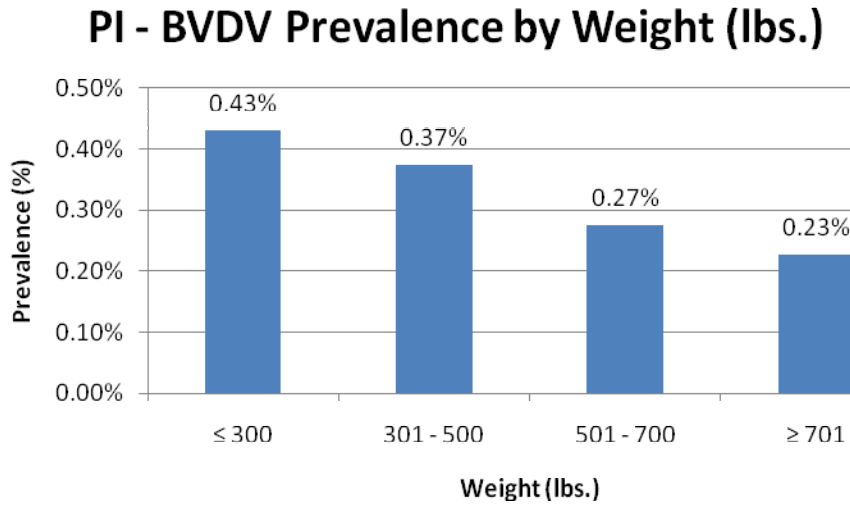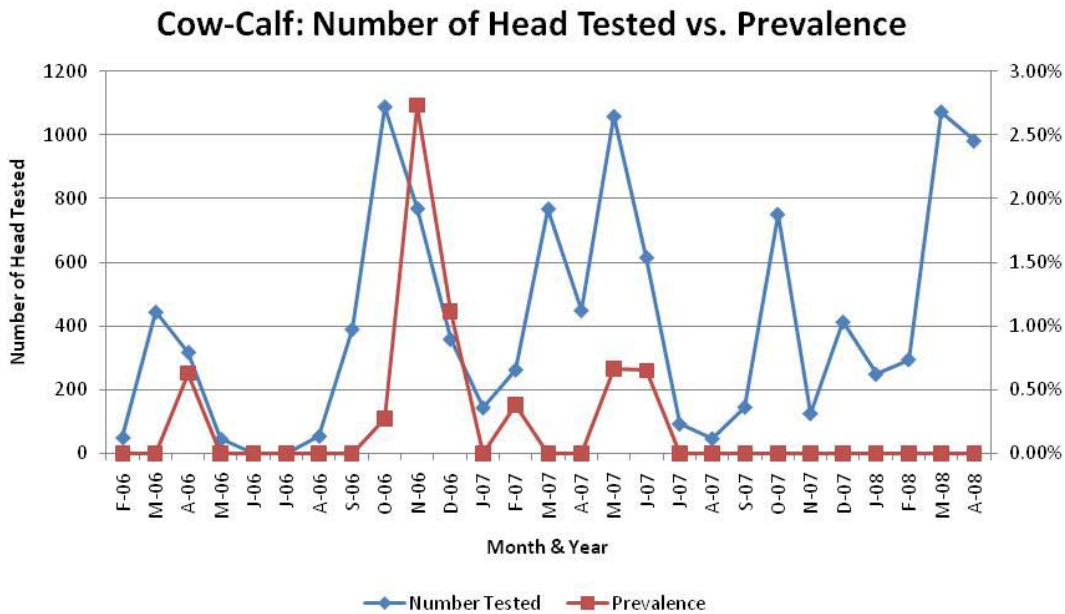

**Figure 5.4.** Number of head tested by month during 2006 – 2008.
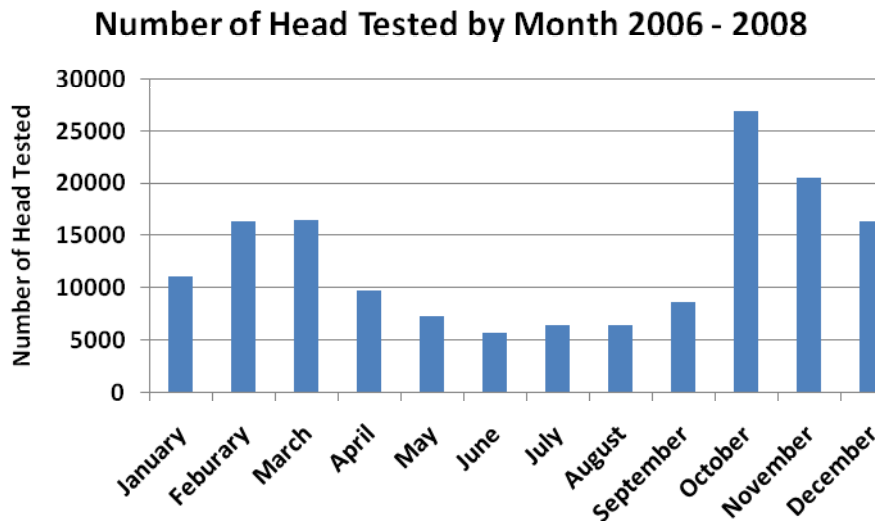
Figure 5.5. Prevalence of PI-BVDV by month of testing from 2006 – 2008.

Figure 5.6. The number of head tested and prevalence of PI individuals when the submitting client was identified as a cow-calf operator.

Figure 5.7. The number of head tested and prevalence of PI individuals when the

submitting client was identified as a stocker operator.
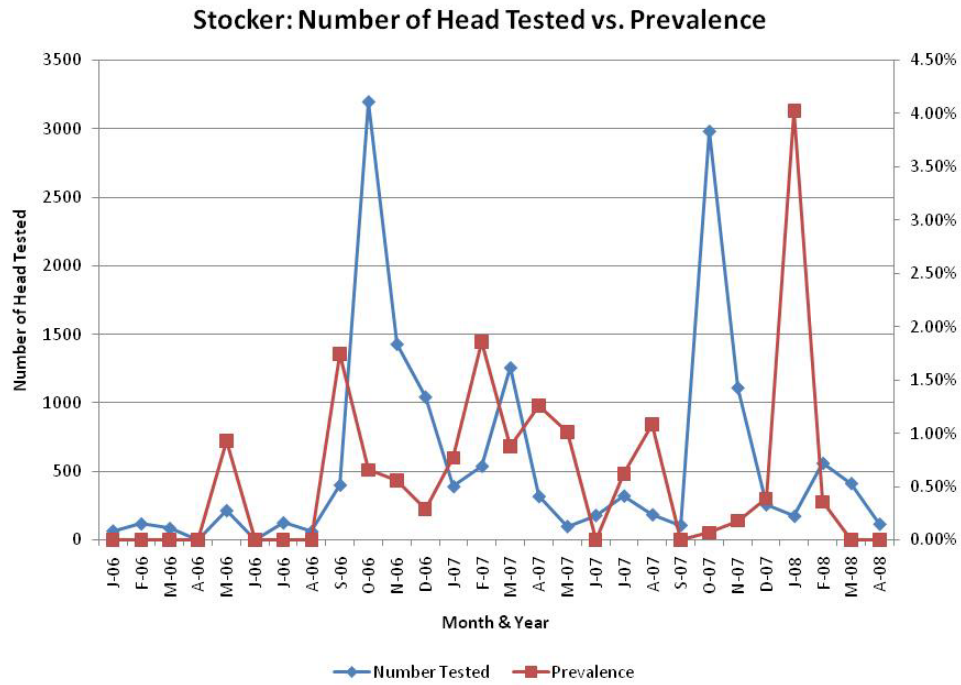
Figure 5.8. The number of head tested and prevalence of PI individuals when the submitting client was identified as a feedlot operator.