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# A Prevalence Study of Southeast Origin Sale Barn Beef Cattle, Comingled in Warren County, Kentucky, Persistently Infected with Bovine Viral Diarrhea Virus, including the Effects of Season and Body Weight

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# A PREVALENCE STUDY OF SOUTHEAST ORIGIN SALE BARN BEEF CATTLE COMINGLED IN WARREN COUNTY, KENTUCKY, PERSISTENTLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS, INCLUDING THE EFFECTS OF SEASON AND BODY WEIGHT

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Presented to
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In Partial Fulfillment
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Master of Science

By
Sarah Elizabeth Thomas

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**A PREVALENCE STUDY OF SOUTHEAST ORIGIN SALE BARN BEEF CATTLE COMINGLED IN WARREN COUNTY, KENTUCKY, PERSISTENTLY INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS, INCLUDING THE EFFECTS OF SEASON AND BODY WEIGHT**

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Bovine viral diarrhea (BVD) is an economically important disease of cattle. Calves persistently infected (PI) with the bovine viral diarrhea virus (BVDV) are a powerful agent for spread of the virus. A total of 24,423 southeast origin beef cattle comingled at three Warren County, Kentucky locations were tested from November 2007 to June 2010 for PI BVDV. A total of 97 head tested positive for PI BVDV, giving an average overall prevalence of 0.397%.

Calves tested were subdivided into categories for additional calculations of dependence. A total of 8,910 were categorized by weight range upon testing (300-399 lbs, 400-499 lbs, 500-599 lbs, and 600-699 lbs). Prevalence does show a dependence on weight, with a higher prevalence found in lower weight classes, especially 300-399 lb calves ($P<0.001$). A total of 24,423 were categorized by season at time of testing (Fall, Winter, Spring, Summer). Prevalence does not show a dependence on season ($P>0.05$).

Although eradication programs are not likely to be organized in the United States, several control programs have been developed. These findings can be used as additional support for PI testing of calves, especially those in lighter weight classes, as part of a BVD control program.

# Chapter One

## Introduction

Bovine Viral Diarrhea (BVD) is a major disease that negatively affects beef and dairy cattle worldwide. The Bovine Viral Diarrhea Virus (BVDV) is a single-stranded RNA virus that is not usually fatal, but has a negative impact because it suppresses the immune system of infected cattle and makes them more susceptible to other diseases (Bolin, Grooms, 2004). Cattle affected by BVDV can be of any age; older cattle may have reproductive failure, abortions, and decreased performance and production, and calves may experience lower weaning weights and rates of gain (van Campen, 2010). BVDV can affect every aspect of a beef operation, from seed-stock, cow-calf, stocker operations, to feedlots (Ridpath, 2009). Because of these conditions, producers experience an overall economic loss when cattle are affected by BVDV.

A major source of BVDV is cattle that are persistently infected (PI). PI calves shed extremely high numbers of virus particles for life, making them a powerful agent for its spread (Fulton, 2005). A calf becomes persistently infected when it is exposed as a fetus to the BVD virus between days 42 and 125 of gestation (Fulton et al., 2009). For a PI calf to become exposed, the dam must be either persistently infected herself, or she must have been exposed to the virus at this period of gestation (Fulton et al., 2009).

BVD is not a new disease in cattle, as it was first identified in the 1940's (Ridpath, 2010). However, because of the development and availability of new tools and diagnostic tests to identify PI animals, there has been an increase of interest in this area and in the possibility of control or eradication of BVD. Diagnostic tests for detection of PI animals include antigen-based testing methods such as antigen-capture ELISA

(Ridpath, 2009). Testing and removing PI animals helps break the BVD cycle and decreases the spread of the virus. Controlling the disease requires identifying and eliminating PI cattle from the herd, maintaining good records, developing a plan to keep PI cattle from entering the herd, and keeping a PI calf from being created in the herd by developing and following a sound vaccination program (Brock et al., 1998).

This study was developed to gain a better understanding of the prevalence of PI beef cattle of southeast US origin comingled in Warren County, KY. It was also developed to determine whether there is a significant relationship or dependence between prevalence and weight classes, or prevalence and season.

## Chapter Two

## Review of Literature

BACKGROUND- HISTORY AND CLASSIFICATION OF BVDV

The first recorded observation of bovine viral diarrhea (BVD) dates to 1946, in a New York dairy (Olafson et al., 1946). The new disease was found to cause leukopenia, pyrexia, depression, diarrhea, anorexia, gastrointestinal erosions, and hemorrhages in cattle (Ridpath, 2010). In 1957, the causative viral agent was isolated and termed the bovine viral diarrhea virus (BVDV) (Lee et al., 1957).

Along with classical swine fever virus (CSFV) found in hogs and border disease virus (BDV) found in sheep, BVDV is currently classified as a member of the genus *Pestivirus* within the family *Flaviviridae* (Fan, Wang, 2009). BVDV is a single-stranded, positive-sense RNA virus, and is classified based on genotype and biotype (Fray et al., 2000).

The two genotypes, BVDV1 and BVDV2, are based on phylogenetic analysis of differences in the viral genome, and also on antigenic differences; antigenic differences are shown by differences in cross-neutralization and monoclonal antibody binding patterns (Bachofen et al., 2010; Fulton et al., 2006; Ridpath, 2010). The BVDV1 and BVDV2 genotypes can be further divided into subgenotypes: 12 subgenotype groupings within the BVDV1 species (BVDV1a, BVDV1b, etc) and two subgenotype groupings within the BVDV2 species (BVDV2a and BVDV2b) (Ridpath, 2010). Genotypes of the BVDV are about 60% similar to each other at their base sequence, and subgenotypes are about 80% to 85% similar to each other (Bolin, Grooms, 2004). Subgenotypes BVDV1a, BVDV1b, BVDV2a, and BVDV2b are the most common that are found in North

America (Fulton et al., 2006). Predominance of these different BVDV subgenotypes varies according to different geographic locations (Ridpath, 2010). This is accounted for most likely by routes of movement of cattle, vaccine usage, and geographic isolation of cattle populations (Bolin, Grooms, 2004).

Independent of genotype, BVDV strains are also classified by biotype based on their effect on cultured cells. The non-cytopathic (NCP) biotype replicates in cultured cells without inducing cell death, while the cytopathic (CP) biotype induces cytoplasmic vacuolation and cell death of cultured cells within a few days of infection (Fray et al., 2000; Ridpath, 2010). In the field, the NCP biotype is most common, while the CP viruses are more rare (Fray et al., 2000; Ridpath, 2010). CP viruses are usually coisolated with a NCP virus from tissues of cattle with signs of mucosal disease (Bolin et al., 2009; Ridpath, 2010). CP viruses are the result of either homologous or heterologous recombination of the parent NCP viral RNA; reversion of the CP virus back to the NCP biotype may also occur (Bolin, Grooms, 2004; Bachofen et al., 2010).

Like other RNA viruses, the BVDV is able to mutate rapidly (Bolin, Grooms, 2004; Bolin et al., 2009).

## EFFECTS OF BVDV

The BVDV can be spread in a variety of ways, including transmission from acutely infected cattle, by inanimate objects that may carry the virus, bovine sera, rectal examination, fluids used for embryo transfer, infected semen, and contaminated vaccines (Fray et al., 2000).

Following infection with BVDV, depending on the agent, host, and environment, a wide range of outcomes can occur that vary in severity. Factors include "whether the host is immunotolerant or immunocompetent to BVDV, immune status (passive from colostral antibodies or active from exposure or vaccination), pregnancy status in females, gestational age of the fetus at the time of infection, level of environmental stress at the time of infection, and concurrent infection with other pathogens" (Bolin, Grooms, 2004).

The majority of BVDV isolates are of low virulence and induce subclinical to very mild disease; subclinical infections can result in mild fever, leukopenia, and in the majority of unvaccinated cattle, development of serum-neutralizing antibodies (Bolin, Grooms, 2004). It is estimated that 70% to 90% of BVDV infections are subclinical (Ames, 1986).

However, BVDV infections may also lead to clinical disease; in cattle, the BVD virus causes diseases that are termed BVD, mucosal disease, chronic BVD, virulent acute BVD, and hemorrhagic syndrome (Bolin et al., 2009). BVD most commonly results in lethargy, anorexia, fever, diarrhea, and decreased milk production in lactating cows (Bolin, Grooms, 2004). Severe BVD causes high morbidity; this peracute infection is characterized by fever, pneumonia, and sudden death in all age groups of cattle (Carman et al., 1998). Acute BVD infections can lead to hemorrhagic syndrome, which can cause severe thrombocytopenia, bloody diarrhea, epistaxis, hemorrhages on mucosal surfaces, hyphema, bleeding from injection sites, pyrexia, leukopenia, and death (Corapi et al., 1990). Except in the cases of some hypervirulent type 2 BVDV strains that cause the lethal severe acute BVD, most of the BVDV biotypes cause acute infection, and the hosts can effectively clear the virus by their own immunity (Fan, Wang, 2009).

Pestiviruses encode two unique proteins that have an affinity for cells involved in the immune system: a nonstructural protein ($N^{pro}$) that suppresses the host's immune system by preventing production of type I interferon, and an envelope glycoprotein ($E^{rns}$) that prevents the induction of beta interferon by binding to and degrading double-stranded RNA (Ridpath, 2010). As a member of the *Pestivirus* genus, BVDV can cause infection resulting in immunosuppression of the host, increasing its susceptibility to other pathogens and enhancing the pathogenicity of any coinfecting pathogen (Bolin, Grooms, 2004). BVDV infections have been associated with *Mannheimia haemolytica*, bovine herpesvirus-1, bovine respiratory syncytial virus, salmonellosis, *Escherichia coli*, bovine popular stomatitis, rotavirus, and coronavirus infections (Bolin, Grooms, 2004). BVDV has been reported as the most common virus isolated from outbreaks of bovine respiratory disease (BRD) in the United States (Bolin, Grooms, 2004). It usually does not cause disease alone, but can work with *M haemolytica* (Potgieter et al., 1984), bovine herpesvirus (Potgieter et al., 1984), or the bovine respiratory syncytial virus (Broderson, Kelling, 1998) to cause BRD, especially in a stressful environment.

BVDV also has a major effect on reproduction in cattle. In infected bulls, semen quality may decrease, and infected cows may have decreased conception rates, increased early embryonic deaths, abortions, and still-births, and calves may have congenital defects (Bolin, Grooms, 2004; Fray et al., 2000; Bolin et al., 2009). Conception rates may fall by up to 44% (Fray et al., 2000). If the dam becomes exposed to the BVDV during mid-gestation, a higher rate of congenital abnormalities occur, such as alopecia, pulmonary hypoplasia, retarded growth, thymic aplasia, ataxia, cerebellar hypoplasia, CNS defects, and ocular lesions (Fray et al., 2000). If the dam becomes exposed to the

BVDV later in gestation, congenital abnormalities still occur but are less common, and there is still a risk of abortion; however, most calves infected late in gestation are born clinically normal and have high levels of pre-colostral antibodies (Fray et al., 2000).

In regards to spread of the disease, the greatest effect of BVDV occurs when the dam is exposed to the virus early in gestation, between days 42 and 125; through transplacental infection, the fetus can become immunotolerant to and is persistently infected (PI) with the BVDV (Bolin, Grooms, 2004; Fulton et al., 2009).

Type 1 BVDV more often results in PI, congenital defects, and weak calves, and Type 2 BVDV more often results in aborted fetuses (Evermann, Ridpath, 2002).

Costs associated with BVD can vary according to the type of operation. Ridpath (2002) states that BVD can result in costs of $35.00 to $65.00 per calving on a US dairy. Costs on a US beef cow-calf operation can range from $15.33 to $20.16 per cow (Larson et al., 2002), and are $41.17 per head on a US beef feedlot (Hessman, 2006).

## PI CALVES

Only the NCP biotype can cause persistent infection (PI) of the fetus (Bachofen et al., 2010; Bolin et al., 2009). As a result of fetal infection before the onset of immunologic competence, PI calves are immunotolerant to the infecting viral strain, differing from other persistent viral infections in humans and animals (Bachofen et al., 2010; Bolin et al., 2009; Peterhans et al., 2006). Immunotolerance in the PI calf is specific to only the particular infecting BVDV strain (Bolin, Grooms, 2004). The PI calf

remains infected for life and sheds large quantities of the virus (Bachofen et al., 2010). A PI calf sheds one million to 10 million virus particles every day of its life, in comparsion to a BVD animal, which sheds 1,000 to 10,000 virus particles a day for a period of only six to 10 days (IDEXX Laboratories Inc., 2008). Because of the efficiency by which PI animals can spread BVDV, PI animals should be accounted for and included in any control or eradication program.

Calves born PI may either be stunted and weak, or normal in size and in appearance; although some PI calves appear clinically normal, they frequently are poor doers and have a short life span and leave the herd prematurely (Fray et al., 2000; Bolin et al., 2009). PI animals often have chronic intestinal or pulmonary symptoms, and may also have dermatological, neurological, or haematological disorders (Bachofen et al., 2010). Persistent infection not only affects the fetus, but it can also affect the immune response of the dam by leading to down-regulation of important signaling pathways in her blood (Drovers, 2009).

If a PI calf is exposed to a CP BVDV biotype, mucosal disease (MD) may occur (Bolin, Grooms, 2004). Both NCP and CP BVDV biotypes will be isolated in MD, which helps in diagnosis of the disease (Bachofen et al., 2010). Not every combination of NCP and CP virus will result in MD; the CP biotype must be homologous to the persisting NCP biotype (Bolin, Grooms, 2004). MD can occur in both BVDV Type 1 and Type 2 genotypes (Bolin, Grooms, 2004). Modified-live BVDV vaccines and super-infection with the CP BVDV can lead to MD (Bolin, Grooms, 2004). The disease is usually lethal, and causes mucosal lesions, destruction of the lymphoid tissue in the gastrointestinal tract, and untreatable diarrhea (Bachofen et al., 2010).

PI calves have an effect on every sector of the beef cattle industry. After exposure of the susceptible nonvaccinated penmates to a PI calf in a feedlot situation, 70% to 100% become infected with BVDV (Fulton et al., 2005; Fulton et al., 2006). According to Loneragan and colleagues (2005), 15.9% of initial cases of BRD are attributable to exposure to PI calves. According to van Campen (2010), feedlots are the endpoint for the BVD virus in terms of transmission, but the economic effect is easily seen on feeder calves, which accounts for strong interest in control of BVDV in this segment of the industry.

In cow-calf operations, synchronous, seasonal breeding is common, and, depending on the time of exposure, contact with a single PI calf through shared pasture can have significant detrimental effects on the pregnant cows: there may be an increase in infertility, abortions, stillbirths, and birth of calves that are weak or stunted (van Campen, 2010). If the dam is exposed to the BVDV and a PI calf is created, but it is either not born or dies before it is able to infect the breeding herd, there is no sustained effect of BVDV infection on the herd (van Campen, 2010). However, if the PI calf survives into the breeding season, the herd may enter an endemic state of infection (van Campen, 2010). After PI exposure and BVDV infection of the breeding herd, up to 50% of the calf crop may be lost (van Campen, 2010). Of those exposed calves that survive, many will experience diarrhea and pneumonia after maternal antibodies wane, and will also have lower weaning weights and lower rates of gain (van Campen, 2010).

In addition to shared pastures, high risk practices in the beef industry also include heifer development feedlots and the purchase of untested cattle and pregnant heifers (van Campen, 2010). Purchased cattle also often pass through multiple sales facilities, or may

be purchased from multiple sources and then comingled at a high animal density (van Campen, 2010).

DETECTION OF PI CALVES/ DIAGNOSTIC TOOLS

Since persistently infected animals are a continuous source of the BVDV, the identification and removal of PI animals is an important part of any prevention or control program (Brock et al., 1998). After the wane of maternal antibodies, PI calves usually have detectable amounts of the NCP virus in their serum, with concentrations of $10^4$- $10^6$ $CCID_{50}$/ mL of serum (Brock et al., 1998). A variety of diagnostic tools are available that may target the viral antigens (ex: immunoperoxidase microtiter assay, antigen-capture [Ag]ELISA, immunochemistry [IHC], fluorescent antibody), genomic material (ex: traditional and real-time reverse transcription PCR, in situ hybridization), or BVDV specific antibodies (ex: virus neutralization, antibody ELISA) (Bolin, Grooms, 2004; Brock et al., 1998).

In antigen detection assays, either monoclonal or polyclonal antibodies are used to detect BVDV antigens (Bolin, Grooms, 2004). Monoclonal antibodies are specific for a single epitope, and binding of the antibody may not occur if there is any epitope variation between viruses (Bolin, Grooms, 2004). However, polyclonal antibodies react with multiple epitopes, and these are often conserved among viruses (Bolin, Grooms, 2004). Therefore, "most antigen detection assays use polyclonal antibodies or a pool of monoclonal antibodies to provide the broadest reactivity and capability of detecting a diverse population of BVDV isolates" (Bolin, Grooms, 2004).

Antigen detection assays such as IHC and AgELISA are very accurate and cost effective at detecting both BVDV-infected calves and PI calves (Cornish et al., 2005). Although tested individually, large numbers of animals can be tested without difficulty because samples can be easily collected when calves are processed or handled, either by obtaining ear notches or blood samples (Cornish et al., 2005). According to Cornish and colleagues (2005), the IHC test provides results in 5 days, while the more time-efficient and less labor-involved AgELISA test can provide results in as soon as one day. Since both acute and persistent infection result in a positive diagnosis, a positive animal should be tested again 30 days after the initial test to make a final diagnosis (Cornish et al., 2005). Testing is also cost-efficient, as the AgELISA test offered through IDEXX (HerdChek BVD Antigen Test Kit) costs less than $5 per head (IDEXX, 2008). The IDEXX HerdChek BVD Antigen Test Kit is a USDA-licensed test based on the $E^{rns}$ (gp48) antigen, and it detects both type 1 and type 2 BVDV, with 100% sensitivity and 100% specificity on skin samples (IDEXX, 2008).

Edmonson and colleagues (2007) state that isolation of the viral antigen from serum and then identification of the viral isolate by immunofluorescence or immunoperoxidase monolayer assay is one of the most reliable diagnostic techniques.

Polymerase chain reaction (PCR) detects and amplifies the viral genomic sequence (Bolin, Grooms, 2004). Pooling of samples is common when using PCR as a diagnostic tool; when determining the size of the pool, the pooling protocol should use the fewest number of tests required to identify all animals PI with BVDV in a herd (Edmonson et al., 2007). According to Edmonson and colleagues (2007), there is an inverse ratio between prevalence and pool size: the optimum number of samples in an

initial pool would be 20 to 30 for a PI prevalence of 0.5% to 1.0%, and as prevalence increases the least-cost initial pool size decreases.

## CONTROL OF DISEASE

Testing is the only way to correctly identify PI calves, as they are not always identifiable based on sight. Therefore, testing and removal of PI animals should be an important part of any control program (Brock et al., 1998).

Vaccination is also an important facet of control; an estimated 80% of cattle in the US are vaccinated with either inactivated or modified live viral vaccines (MLV) containing BVDV (USDA, 1995; USDA, 2007). Control programs include the use of inactivated and MLV BVDV vaccines to prevent fetal infections, reproductive losses, and acute infections (van Campen, 2010). Prior to 2004, vaccines contained cytopathic Type 1a BVDV (either Singer or NADL), but after the recognition that Type 2 BVDV can lead to severe disease and fetal losses, a cytopathic Type 2 BVDV was also included in many vaccines (van Campen, 2010). MLV vaccines have been shown to offer superior protection, but there are still some concerns about their safety and effects on health (van Campen, 2010).

Vaccine failure is most common and fetal protection is most limited when the challenge virus is a different genotype than the vaccine virus (Bolin et al., 2009). Several studies do show vaccination with Type 1 BVDV does induce some clinical cross protection against Type 2 challenge (Bolin, Grooms, 2004). However, Drovers (2009) cites a study in which Fulton and colleagues found that the majority of viral isolates from

PI cattle were of the BVDV Type 1b subtype, while current USDA approved vaccines primarily contain BVDV Type 1a and Type 2a, suggesting that complete protection is not offered. Vaccination of feeder calves is beneficial, but to gain complete protection, ideally, vaccination should also include the breeding herd for control of reproductive failure and prevention of fetal infection, and the subsequent birth of PI calves (Bolin, Grooms, 2004; Fernandez et al., 2009). Regarding fetal protection, complete cross protection may not be provided from current vaccines; according to Bolin and Grooms (2004), experimental fetal protection trials have ranged in efficacy from 25% to 100%, depending on the type of challenge virus and vaccine virus.

Vaccine strategies are varied, and many focus on vaccination at critical periods of production (Bolin et al., 2009). However, vaccines are often not given consistently or appropriately (Carruthers, Petrie, 1996). According to Bolin and colleagues (2009), three assumptions are often made that may lead to failure of a vaccine program; these assumptions are that the vaccine was handled properly before vaccination, that the herd was appropriately vaccinated, and that all cattle within the herd have an equal immune response to the vaccine. Producers that do not take these assumptions into account may experience apparent vaccine failure (van Campen, 2010).

Any control program that includes proper vaccination and testing should also include strict biosecurity measures to sustain a BVDV-free population (Fray et al., 2000). The number of BVD control programs has increased since 2003, when the Academy of Veterinary Consultants published a position statement (http://www.avc-beef.org/links/BVDLinks.asp) on the control and possible eradication of BVD in the United States (van Campen, 2010). According to van Campen (2010), as of 2010 there

are beef control programs in Alabama, Colorado, Georgia, Mississippi, Montana, Oregon, and Washington, and a dairy control program in New York. The current control programs are all voluntary, associated with a university, and work along with other beef and dairy quality assurance programs (van Campen, 2010). The control programs include "education about BVDV transmission and diseases, required testing procedures, documentation of biosecurity practices to prevent re-introduction of BVDV, and verified use of a vaccination schedule" (van Campen, 2010).

Although mandatory BVD control programs exist in several European countries, there are not currently any mandatory programs in place in the United States (van Campen, 2010). Several obstacles exist, including the need for a control program to be government-regulated, and the belief that there is a lack of clear danger, based on low herd prevalences (van Campen, 2010).

# Chapter Three

## Materials and Methods

As an initial step, in the fall of 2009, a visit was made with Sandy Grant at the Gold Standard diagnostic lab in Bowling Green, KY, to discuss the Bovine Viral Diarrhea Virus and the methods used at the lab for testing persistently infected (PI) cattle. Several additional visits were conducted to gain experience in running the AgELISA diagnostic test (IDEXX HerdChek BVD Antigen Test Kit) used at this location. Also in the fall of 2009, a visit was made to Farm A in Warren County, KY, to gain knowledge on obtaining ear-notch and serum samples. PI test results (positive or negative) from November 2007 to June 2010 were obtained from the herd veterinarian in Bowling Green, KY on beef cattle from Farms A and B. Additional test results and weight range of those tested were obtained from Farm C. Data for this study were obtained first hand by collecting ear-notch and serum samples, then testing them at Gold Standard diagnostic laboratory, and also by obtaining previous and current testing records. Records were obtained from Farms A and B herd veterinarian for positive or negative test results on cattle from November 2007 to June 2010. Records were obtained from Farm C diagnostic lab for results from April 2009 to June 2010.

Study animals from each beef operation were of Kentucky, Tennessee, or Alabama origin, and ranged from 300 to 600 pounds when processed upon arrival. All the calves were assumed to be naïve to vaccination when initially processed. All calves that were purchased and processed were tested to determine if they were persistently infected with the bovine viral diarrhea virus.

Ear notching was the most common method by which samples for testing were obtained. Calves were restrained in a chute and, using V-type ear-notchers, a full notch was taken from the top portion of the ear to reduce the amount of hair. The notch was placed in a sample vial containing enough phosphate buffered solution (PBS) for the sample to be submerged. The sample vial was labeled, and the corresponding animal ID's and sample vial ID's were recorded. To reduce spread of disease and to decrease chances of inaccurate test results, the ear notchers were then rinsed in a clean water vessel to remove any hair, then disinfected in a vessel containing diluted chlorhexidine (1 ounce chlorhexidine: 1 gallon water), and then in another clean water vessel to rinse remaining disinfectant. After all the ear-notch samples were gathered, the sample vials were immediately boxed in trays along with cold packs and lab submission forms and sent to the appropriate lab for diagnostic testing. Samples not sent out were refrigerated and then sent as soon as possible.

Blood collection was a less common method by which samples for testing were obtained. While the calf was restrained in the chute, a 3 cc blood sample from either the neck or the tail was collected in a marble top tube. The sample was centrifuged and the serum was sent to the appropriate diagnostic lab for testing.

Samples from Farms A and B were tested for PI BVDV with an antigen capture ELISA test at the Elizabethtown, KY, Central States Testing diagnostic lab location.

Samples from Farm C were tested for PI BVDV with an antigen capture ELISA test at the Bowling Green, KY, Gold Standard diagnostic lab location.

Results from the diagnostic labs were available within as few as 5 hours.

Data from Farms A, B, and C were compiled in a Microsoft Excel spreadsheet and organized by specific location, date, total number tested, and number testing positive and negative.

Data from Farm C were also grouped by weight range at time of testing.

## Chapter Four

## Results

All data obtained from beef cattle testing positive or negative for persistent infection with BVDV from November 2007 to June 2010 are listed by date and location in Table 1. A total of 97 out of 24,423 tested positive as PI BVD, giving an overall prevalence of 0.397%.

**Table 1. Beef cattle testing positive for PI BVD by location (Nov 2007 to June 2010).**

| Location | Total number tested | Total number positive | Prevalence (%) |
|---|---|---|---|
| FARM A | 9271 | 39 | 0.421 |
| FARM B | 6242 | 24 | 0.384 |
| FARM C | 8910 | 34 | 0.382 |
| TOTAL | 24,423 | 97 | 0.397 |

Data obtained from Farm C cattle testing positive or negative for persistent infection with BVDV from April 2009 to June 2010 are listed by weight range in Table 2. Calves were grouped by weight range regardless of season.

**Table 2. Farm C- beef cattle testing positive for PI BVD by weight range (April 2009 to June 2010).**

| Weight range (lbs) | Total number tested | Total number positive | Prevalence (%) |
|---|---|---|---|
| 300-399 | 1491 | 15 | 1.006 |
| 400-499 | 3283 | 9 | 0.274 |
| 500-599 | 3694 | 9 | 0.244 |
| 600-699 | 442 | 1 | 0.226 |

Data obtained from beef cattle at all three farm locations testing positive for PI BVD from November 2007 to June 2010 are listed by season in Table 3.

**Table 3. Beef cattle testing positive for PI BVD by season (Nov 2007 to June 2010).**

| **Season** | **Total number tested** | **Total number positive** | **Prevalence (%)** |
|---|---|---|---|
| Fall (Sept 22- Dec 21) | 7419 | 28 | 0.377 |
| Winter (Dec 22- March 21) | 4459 | 15 | 0.336 |
| Spring (March 22- June 21) | 8189 | 36 | 0.440 |
| Summer (June 22- Sept 21) | 4356 | 18 | 0.413 |

A contingency chi-square was used to determine if prevalence of PI BVDV has dependence on weight (Table 4). The calculated $\chi^2$ value of 18.362 is greater than critical $\chi^2$ values at the 0.05, 0.01, and 0.001 levels: 7.815, 11.345, and 16.266, respectively. Therefore, the null hypothesis is rejected at the 0.001 level; frequency (prevalence) does depend on category (weight), and the major contributor to the outcome is in the first category (300-399 lbs).

**Table 4. Contingency Chi-Square; Farm C beef cattle testing positive for PI BVD by weight.**

| **Weight range (lbs)** | | **Positive** | **Negative** | **Total** |
|---|---|---|---|---|
| 300-399 | O | 15 | 1476 | 1491 |
| | E | 5.69 | 1485.31 | |
| | $(O-E)^2/E$ | 15.23 | 0.06 | |
| 400-499 | O | 9 | 3274 | 3283 |
| | E | 12.53 | 3270.5 | |
| | $(O-E)^2/E$ | 0.94 | 0.004 | |
| 500-599 | O | 9 | 3685 | 3694 |
| | E | 14.10 | 3679.90 | |
| | $(O-E)^2/E$ | 1.84 | 0.007 | |
| 600-699 | O | 1 | 441 | 442 |
| | E | 1.69 | 440.31 | |
| | $(O-E)^2/E$ | 0.28 | 0.001 | |
| **Total** | | 34 | 8876 | 8910 |

$\chi^2 = \sum (O-E)^2/E$

calculated $\chi^2 = 18.362$; df = 3

critical $\chi^2$ (0.05, 3) = 7.815

critical $\chi^2$ (0.01, 3) = 11.345

critical $\chi^2$ (0.001, 3) = 16.266

In the contingency chi-square, O= observed frequency and E= expected frequency in each classification. E is calculated by multiplying the respective row total by the respective column total and dividing by the overall total. The 15.23 component of the $\chi^2$

for the 300-399 lbs positive classification= 34 x 1491/ 8910= 5.69; $(15-5.69)^2/ 5.69=$ 15.23. The sum of the $(O- E)^2/ E$ values for all the classification combinations= $\chi^2$. A significant $\chi^2$ indicates dependence between the variables.

A second contingency chi-square was used to determine if prevalence of PI BVDV has dependence on season (Table 5). Since the calculated $\chi^2$ value of 0.8909 is less than the critical $\chi^2$ value of 7.815, the null hypothesis is accepted at the 0.05 level; frequency (prevalence) does not depend on category (season).

**Table 5. Contingency Chi-Square; Beef cattle testing positive for PI BVD by season.**

| **Season** | | **Positive** | **Negative** | **Total** |
|---|---|---|---|---|
| Fall | O | 28 | 7391 | 7419 |
| | E | 29.466 | 7389.534 | |
| | $(O-E)^2/ E$ | 0.0729 | 0.0003 | |
| Winter | O | 15 | 4444 | 4459 |
| | E | 17.710 | 4441.290 | |
| | $(O-E)^2/ E$ | 0.4147 | 0.0017 | |
| Spring | O | 36 | 8153 | 8189 |
| | E | 32.524 | 8156.476 | |
| | $(O-E)^2/ E$ | 0.3715 | 0.0015 | |
| Summer | O | 18 | 4338 | 4356 |
| | E | 17.301 | 4338.699 | |
| | $(O-E)^2/ E$ | 0.0282 | 0.001 | |
| **Total** | | 97 | 24326 | 24423 |

$\chi^2 = \sum (O-E)^2/ E$

calculated $\chi^2 = 0.8909$

df = 3

critical $\chi^2$ (0.05, 3) = 7.815

## Chapter Five

## Discussion

Results from this study may be used as additional support for PI testing as part of a BVDV control program. Data show an overall prevalence of 0.397% of sale barn animals as persistently infected with BVDV. Although prevalence does not depend on season, there is a higher prevalence of PI BVDV in lighter weight calves, especially those ranging in weight from 300 to 399 pounds ($P<0.001$). Although there is a seemingly low prevalence, these PI calves have a significant detrimental impact on other animals they come in contact with as a result of shedding abnormally high numbers of BVDV particles. Although the feedlot is an endpoint in terms of virus transmission, BVDV still has a great economic effect on this segment of the industry. Producers may feel that there is not an even trade-off between costs and benefits of testing, but data from this study show a uniform prevalence, and a testing and control program would be very beneficial.

Although the development of a government funded eradication program is not likely in the United States, the control of BVD is nevertheless important, and several control programs are currently in place. A major concern of any control program should include testing for and removal of any calves that are PI, and maintaining accurate records and documentation of testing. Once an animal has been tested PI negative, there is no need to retest it for PI. PI animals should be removed from the herd; they may be either humanely slaughtered, or grouped together in an isolated pen to prevent the spread of the virus, and then raised to market weight. A vaccination and testing program should also be developed that is specific for the type of operation, and vaccines should be given correctly. Producer failure appears to be more often at fault than vaccination failure.

Based on data from this study, PI testing and removal would be beneficial, and is especially justified in lighter weight sale barn calves.

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