**Effect of maternally-derived immunity in the development of humoral immune responses to vaccination and subsequent challenge with BVDV**

by

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## Abstract

Bovine viral diarrhea virus (BVDV) is a viral pathogen of cattle that can present with different clinical syndromes including reproductive failure, immunosuppression, respiratory disease, and gastrointestinal disease. Significant economic losses have been associated with the clinical presentation of BVDV in cattle operations worldwide. BVDV has been identified as an important contributor in the bovine respiratory disease complex (BRDC) in calves due to its ability to cause immunosuppression and act in synergism with other viruses and bacteria. Vaccination of young calves against BVDV is considered an important management practice to prevent losses associated with acute BVDV infection and BRDC.

Previous research has demonstrated that maternally-derived antibodies to BVDV can protect against acute BVDV infection. When maternal antibodies start to decay and disappear, calves become susceptible to infection and disease; however, the duration of maternally-derived immunity is variable among young calves and some calves become susceptible to BVDV infection earlier in life than others. Additionally, the presence of maternally-derived antibodies in calves at vaccination interferes with the induction of adequate antibody responses and can affect the prevention of viremia and BVDV shedding. This research is concentrated on the evaluation of duration of maternally-derived immunity to BVDV and other respiratory viruses and their effect on humoral immune responses to vaccination in young calves.

In the initial study, the duration and decay of specific antibodies to BVDV 1, BVDV 2, bovine herpesvirus 1 (BoHV-1), bovine respiratory syncytial virus (BRSV) and bovine parainfluenza 3 virus (PI3V) was evaluated in dairy calves that received maternal colostrum or a colostrum replacement product at birth. The duration of maternal immunity against BVDV and the other respiratory viruses in calves that received maternal colostrum was significantly variable. Additionally, a higher proportion of calves in the maternal colostrum group became seronegative to BVDV 1 and BVDV 2 earlier in life. In contrast, calves that received the colostrum replacement product had a more uniform and prolonged duration of maternally-derived immunity to BVDV 1 and BVDV 2.

In a second study, early weaned beef calves that received maternal colostrum naturally from their dams and with different levels of maternally-derived immunity to BVDV were vaccinated at weaning at a median age of 72.2 days with three different multivalent, modified live virus (MLV) vaccines containing BVDV 1 and BVDV 2. Forty-five days after vaccination all calves were challenged with BVDV 2. The proportion of calves that seroconverted after vaccination was minimal; however, differences in the ability of vaccines to induce antibody responses were detected. Calves with higher antibody titers to BVDV1 and BVDV 2 before challenge had a lower proportion of calves that became viremic and shed BVDV in nasal secretions compared with controls.

In a third study, early weaned beef calves that received maternal colostrum naturally from their dams and with different levels of maternally-derived immunity to BVDV and BoHV-1 were vaccinated at weaning at a median age of 93.5 days with four

different multivalent, modified live virus (MLV) vaccines containing BVDV 1, BVDV 2, and BoHV-1. Forty-five days after vaccination calves were simultaneously exposed to 6 cattle persistently infected (PI) with BVDV and 8 calves acutely infected with BoHV-1. Differences in the ability of vaccines to induce antibody responses to BVDV but not to BoHV-1 were detected; however, clinical disease was not observed among calves after exposure to BVDV and BoHV-1. Groups with higher mean antibody responses to BVDV 1 and BVDV 2 after vaccination demonstrated a decreased proportion of calves with viremia and BVDV shedding compared with groups with lower mean antibody responses to BVDV 1 and BVDV 2. Viremia or BoHV-1 nasal shedding was not detected among calves at any time during the study.

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| | |
|---|---|
| AEA | Apparent efficiency of absorption of IgG |
| BoHV-1 | bovine herpesvirus 1 |
| BRSV | bovine respiratory syncytial virus |
| BVDV | bovine viral diarrhea virus |
| CBC | complete blood count |
| $CCID_{50}$ | 50% cell culture infective dose |
| CP | cytopathic |
| CR | colostrum replacement |
| CPE | cytopathogenic effects |
| EU | ELISA units |
| GM | geometric mean |
| IN | Intranasal |
| MC | Maternal colostrum |
| MDBK | Madin-Darby Bovine Kidney (cells) |
| MEM | minimum essential medium |
| NCP | noncytopathic |
| PCR | polymerase chain reaction |
| PI | persistently infected |
| PI3V | parainfluenza 3 virus |

| | |
|---|---|
| qPCR | quantitative polymerase chain reaction |
| RT-PCR | reverse transcription polymerase chain reaction |
| SC | subcutaneous |
| SEM | standard error of the mean |
| SN | serum neutralization |
| VI | virus isolation |
| VN | virus neutralization |

# Introduction

Disease in cattle as a result of bovine viral diarrhea virus (BVDV) occurs worldwide and is responsible for considerable economic losses in the cattle industry. Bovine viral diarrhea virus is an important cause of respiratory, enteric, and reproductive disease in cattle. Additionally, it is considered a major player in the presentation of bovine respiratory disease complex (BRDC) in cattle due to its ability to cause immunosuppression and act synergistically with other pathogens to cause disease (Houe 1995; Fulton et al. 2002). Several countries have developed and implemented control or eradication programs for bovine viral diarrhea virus in cattle (Ridpath 2010). For successful control of BVDV, the viral ecology must be fully understood, which includes identifying reservoirs and main sources for viral transmission and infection. In the case of BVDV, persistently infected (PI) cattle that shed continuously large amounts of virus are considered the most important source of BVDV for susceptible cattle (Houe 1999; Smith et al. 2004). Therefore the identification and elimination of cattle persistently infected with BVDV is a key factor in the development of any prevention and control program in beef and dairy operations. In addition to identification and elimination of PI cattle, the establishment of biosecurity measures that prevent contact and transmission of BVDV between infected and susceptible cattle and the establishment of BVDV vaccination

programs to enhance the immunity in the herd are considered the main aspects in the prevention and control of BVDV in cattle (Walz et al. 2010).

Vaccination of cattle against BVDV is considered an important strategy of herd health programs to minimize clinical disease and reproductive losses associated with BVDV infection (Givens et al. 2012; Ridpath 2013). Additionally, vaccination of young calves against common respiratory viruses including BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V has been associated with a reduced mortality and economic losses due to BRDC in beef herds (Fulton et al. 2002; Step et al. 2009). Some studies have demonstrated that at feedlot entry, calves with high levels of serum neutralizing antibodies to respiratory viruses including BVDV 1 and BVDV 2 presented lower BRDC morbidity rates and reduced treatment costs compared with calves with lower level of humoral immunity (Martin et al. 1999; Fulton et al. 2002). Neutralizing antibodies against BVDV obtained from maternal colostrum or vaccination protect against acute clinical disease caused by BVDV infection; however, the presence of maternally-derived immunity adversely affects serologic responses to vaccination due to interference exerted by BVDV-specific maternal antibodies (Ridpath et al. 2003; Zimmerman et al. 2006). The duration and persistency of maternally-derived immunity depends in great extent on the titer of antibodies absorbed from maternal colostrum and their rate of decay over the first weeks of life of the calf. The higher are the antibody titers after colostrum intake the longer the duration of passive immunity and the higher the interference to vaccination (Munoz-Zanzi et al. 2002). Additionally, variability has been reported in the initial range of antibodies to BVDV and other respiratory viruses in calves that receive maternal colostrum at birth (Kirkpatrick et al. 2001; Munoz-Zanzi et al. 2002; Fulton et al. 2004).

It is unknown if commercial colostrum replacement products provide a more consistent and uniform levels of antibody titers to BVDV and other respiratory viruses. It is possible that due to hyperimmunization of colostrum donor cows during the process of production, colostrum replacement products would provide a more uniform level of virus-neutralizing antibodies to respiratory viruses compared to maternal colostrum.

The inconsistency of antibody titers to BVDV and other respiratory viruses among calves after maternal colostrum intake leads to variability in the duration of maternally-derived immunity. Therefore the ability of calves to establish adequate humoral responses to vaccination in the face of maternal antibodies (IFOMA) should be expected also to be variable as some calves with very high antibody titers will not respond with antibody production to vaccination and other with lower antibody titers might respond or allow priming of specific B cell responses. This makes very difficult to predict the best age to vaccinate young calves IFOMA against BVDV and other respiratory viruses (Kirkpatrick et al., 2001; Munoz-Zanzi et al., 2002; Fulton et al. 2004).

Seroconversion or a four-fold increase in antibody titers following vaccination is an accepted method to evaluate response to BVDV vaccination in cattle (Kelling 2004); however, the absence of antibody responses to vaccination did not prevent protection against clinical disease following experimental challenge with BVDV in calves previously vaccinated IFOMA in various studies (Ridpath et al. 2003; Zimmerman et al. 2006, Platt et al. 2009). The priming of naive B and T cells and the induction of specific cell mediated immune memory responses by vaccination IFOMA have been identified as the main source of protection of young calves vaccinated in the presence of maternally-

derived immunity and subsequently challenged with virulent BVDV when maternal antibodies have already decayed (Ridpath et al. 2003; Stevens et al. 2011). A recent study demonstrated that a single dose of a modified-live virus (MLV) vaccine containing BVDV 1 and BVDV 2 is effective protecting calves with different levels of maternally-derived immunity against challenge with virulent BVDV 2 later in life (Platt et al. 2009).

Vaccination of young calves against BVDV should prevent clinical disease and limit virus spread and transmission through decreasing nasal shedding (Ridpath 2013). Several studies evaluating efficacy of BVDV vaccination of young calves IFOMA have demonstrated clinical protection; however, reduction of viremia and virus shedding has been inconsistent (Zimmerman et al. 2009; Stevens et al. 2011). Prevention of viremia and virus shedding is a desirable outcome of BVDV vaccination of young calves that are shipped/marketed to calf rearing facilities, stocker units, or feedlots as it could decrease the amount of infectious virus in the environment and the risk of transmission. Some studies have indicated that adequate priming of B cell responses and antibody production by BVDV vaccination in calves could be associated with a decreased risk of viremia and nasal shedding at challenge (Howard et al. 1992; Peters et al. 2004).

The research described in this dissertation evaluates the persistency of maternally-derived antibodies to respiratory viruses including BVDV 1 and BVDV 2 in calves that receive maternal colostrum or a commercial colostrum replacement product. Additionally, this research analyses the antibody responses, presence of viremia, virus shedding, and clinical disease in young calves with different levels of maternally-derived immunity to BVDV and BoHV-1 following vaccination with multivalent MLV vaccines

containing BVDV and BoHV-1 and subsequently challenged with BVDV or challenged exposed with BVDV and BoHV-1.

# Literature Review

## Bovine Viral Diarrhea Virus

### *Introduction and history*

Bovine viral diarrhea was first described in 1946 after an outbreak of severe diarrhea that affected cattle herds in the state of New York, USA and in the province of Saskatchewan, Canada (Childs 1946; Olafson et al. 1946). The first reports on bovine viral diarrhea described an acute and severe condition, but also less severe and subacute cases were reported (Childs 1946; Olafson et al. 1946). Additional clinical signs described in affected cattle included signs of systemic illness such as depression, fever, and dehydration. Respiratory signs were reported in these outbreaks and included bilateral nasal discharge and elevated respiratory rates. Abortions and dramatic decreases in milk production were also reported (Olafson et al. 1946). Leukopenia was a consistent laboratory abnormality in affected animals. Ulceration and hemorrhage of mucous membranes of the gastrointestinal tract were described at postmortem examination of affected animals. Hemorrhages were also found in other tissues such as the epicardium and vaginal mucosa (Olafson et al. 1946). The disease was first termed "X disease" and subsequently "virus diarrhea" due to suspicions of a viral etiology for this new condition (Childs 1946; Olafson et al. 1946).

Eight years after the first report of this new transmissible disease of cattle, a viral etiology was confirmed (Baker et al. 1954). Also, a similar virus was subsequently associated with a new sporadically-occurring disease termed "mucosal disease" (Ramsey et al. 1953). Contrary to the first descriptions of bovine viral diarrhea, mucosal disease presented with low case attack rates and high case fatality rates (Ramsey et al. 1953). Clinical manifestations of mucosal disease were more severe than bovine viral diarrhea and included severe bloody diarrhea, erosions of mucous membranes, erosions of other epithelial surfaces, and profuse salivation (Ramsey et al. 1953). Viral agents causing clinical cases of bovine viral diarrhea were isolated, and their effects in cell cultures were evaluated (Lee et al. 1957; Underdahl et al. 1957). Initial descriptions of the viral agents indicated that 2 forms existed, one that induced cytopathic effects (cell vacuolation and death) and one that was noncytopathic to cell cultures (Gillespie 1961). The viruses isolated from cases of bovine viral diarrhea were noncytopathic whereas the viruses isolated from cases of mucosal disease were cytopathic (Gillespie 1961). This led to the first classification of BVDV into cytopathic and noncytopathic biotypes. The discovery of different BVDV biotypes would initiate one of the most studied topics on BVDV infection, the pathogenesis of persistent infection and mucosal disease. Persistent infection was determined to be the result of an *in utero* infection with a noncytopathic strain of BVDV before 100 days of gestation (Liess 1984; McClurkin 1984). Studies determined that cattle persistently infected with noncytopathic BVDV developed mucosal disease after a superinfection with cytopathic BVDV (Bolin 1985; Brownlie 1987). Persistently infected animals are currently considered the most important reservoir and source of transmission of BVDV within and between cattle herds (Houe 1999). The study

of BVDV in the last two decades has rapidly increased the knowledge of the epidemiology and molecular biology of BVDV. A newly found viral isolate associated with severe acute disease and high mortality rates in cattle was determined to be genetically different from the initial BVDV isolate (Pellerin et al. 1994; Carman et al. 1998). The genetic dissimilarity between these isolates promoted the classification of BVDV in two genotypes (1 and 2) (Ridpath et al. 1994; Ridpath 2005).

*Taxonomy and prevalence*

Bovine viral diarrhea viruses 1 and 2 are enveloped, single-stranded, RNA viruses in the genus *Pestivirus* from the family *Flaviviridae* (Ridpath 2005). Other viruses from the genus *Pestivirus* that are of veterinary importance include classical swine fever virus (hog cholera virus) and border disease virus of sheep. Based on viral effects on cell cultures, BVDV strains can be subclassified as cytopathic or noncytopathic, and this is referred to as biotype (Ridpath 2005; Ridpath et al. 2006). Cytoplasmic vacuolation and cell death are observed in cell cultures infected with cytopathic strains, while cell cultures remain intact in the presence of noncytopathic virus (Lee et al. 1957; Underdahl et al. 1957). A third biotype of BVDV has been recently reported (Ridpath et al. 2006). This biotype consists of a subpopulation of noncytopathic strains of BVDV capable of causing cytopathic effects in cultures of lymphocytes *in vitro* (Ridpath et al. 2006). The classification of BVDV by biotype is not related with virulence and pathogenicity of the virus *in vivo* (Fulton et al. 2000; Fulton et al. 2005a). Cytopathic, noncytopathic, and lymphocytopathic strains can be equally pathogenic and capable of causing severe disease; however, only noncytopathic strains are able to produce persistently infected

cattle (Fulton et. al 2000; Fulton et al. 2005a, Ridpath et al. 2006). Noncytopathic strains of BVDV represent between 60 to 90% of the BVDV isolates from diagnostic laboratories, and it is believed that noncytopathic BVDV is the most common biotype in nature (Fulton et al. 2000; Fulton et al. 2005a).

Based upon differences in viral nucleic acid sequences, two genotypes of BVDV have been described, BVDV 1 and BVDV 2 (Ridpath et al. 1994); however, genetic mutations and constant antigenic variation are common within BVDV viruses (Bachofen et al. 2008), and BVDV subgenotypes have been described for each BVDV 1 and BVDV 2 genotypes (Vilcek et al. 2005). Twelve subgenotypes have been reported among BVDV 1 (BVDV 1a through BVDV 11) and 2 subgenotypes among BVDV 2 (BVDV 2a and BVDV 2b) (Vilcek et al. 2005). The distribution of BVDV subtypes within the United States cattle population involves 3 major subtypes, BVDV 1a, BVDV 1b, and BVDV 2a. Bovine viral diarrhea virus 1b is the predominating subtype found in the United States and accounts for 78% of persistently infected cattle in some studies in North America (Fulton et al. 2005a; Fulton et al. 2006).

The natural host for BVDV is cattle, and prevalence of seropositive animals is influenced by management conditions, vaccination status, and the presence of persistently infected cattle (Houe 1995; Houe 1999). Serosurveys performed in cattle in North America have demonstrated seropositive rates between 40 and 90%. The percentage of herds with unvaccinated cattle yet seropositive to BVDV ranges from 28 to 53% depending on the geographic location of the herd (Durham et al. 1990; VanLeeuwen et al. 2005; Scott et al. 2006; VanLeeuwen et al. 2006). Prevalence of persistently infected cattle is considerably lower compared with seropositive rates found in individual animals in different studies

and is estimated to be less than 1% of the cattle population (Wittum et al. 2001). Detection of persistently infected cattle within cow/calf operations and those arriving to feedlots in the United States varies from 0.1 to 0.4% (Wittum et al. 2001; O'Connor et al. 2005).

Although cattle are considered the natural host of BVDV, other species are susceptible to BVDV infection including pigs, sheep, goats, bison, deer, and camelids (Passler et al. 2010); however, implications of transmission between these species are still unknown. It is possible that the presence and identification of persistently infected individuals within heterologous species different from cattle may be of critical importance in the epidemiology of BVDV (Passler et al. 2010).

*Clinical Manifestations*

Bovine viral diarrhea virus infection may result in a variety of clinical manifestations in cattle ranging from subclinical disease to acute fatal disease. Interactions between host, environmental, and viral factors can influence the clinical outcome of BVDV infections in cattle (Baker 1995). Host factors include the immune status, pregnancy status, gestational age of the fetus, and presence of concurrent infections with other pathogens. Environmental factors include severe weather and management-related factors such as commingling and transport. Viral factors include variation of viral biotype, genotype, and antigenicity (Houe 1995; Houe 1999). Both BVDV 1 and BVDV 2 can be associated with a wide range of clinical manifestations in cattle (Baker 1995; Ridpath et al. 2006); however, severe acute BVDV infection and the thrombocytopenic / hemorrhagic syndrome described in North America in the early

1990's were principally associated with BVDV 2 strains (Pellerin et al. 1994; Carman et al. 1998).

Three categories can be used to review the clinical manifestations of BVDV infections: 1) BVDV infection in immunocompetent cattle; 2) BVDV infection in the developing fetus; and 3) BVDV infection in immunotolerant cattle (Baker 1995).

*Infection of immunocompetent cattle*

Postnatal infections with BVDV in cattle that have the ability to respond immunologically to the virus are termed "acute" or "transient" and may vary from subclinical infections to peracute BVDV infection and hemorrhagic syndrome. In general, 70 to 90% of BVDV infections are subclinical or inapparent (Ames et al. 1986); however, animals affected with "inapparent" or subclinical infections can exhibit mild fever, leukopenia, decrease in feed intake, and decrease in milk production (Moerman et al. 1994). Acute BVDV infections are characterized by fever, diarrhea, depression, oculonasal discharge, anorexia, decreased milk production, oral ulcerations, and leukopenia characterized by lymphopenia and neutropenia (Baker 1995).

Peracute BVDV infection reported in beef, dairy, and veal operations in the United States and Canada has been associated with severe clinical signs and higher case fatality rates (Pellerin et al. 1994; Carman et al. 1998). Severe and bloody diarrhea, fever, oral ulcerations, respiratory disease, abortions and decreased milk production have been described in cases of peracute BVDV infection (Pellerin et al. 1994; Carman et al. 1994). Histopathologic lesions of peracute BVDV cases were characterized by severe lymphoid depletion of Peyer's patches, necrosis of intestinal epithelium, and ulcerative lesions in

the alimentary tract (Carman et al. 1998). Genomic analysis of the viral isolates associated with peracute BVDV infections revealed BVDV 2 genotypes (Carman et al. 1998). Hemorrhagic syndrome is another form of severe BVDV infection in cattle associated with noncytopathic isolates of BVDV 2 (Evermann et al. 2005). Clinical manifestations of hemorrhagic syndrome include severe thrombocytopenia, hemorrhage, bloody diarrhea, epistaxis, petechial hemorrhages, ecchymotic hemorrhages, bleeding from injection sites, and death (Rebhun et al. 1989; Bolin et al. 1992). Marked thrombocytopenia of affected cattle is characterized by altered function of platelets, thus quantitative and qualitative platelet defects contribute to the observed hemorrhagic diathesis (Walz et al. 2001). Another important contributing factor to thrombocytopenia induced by BVDV is the viral infection of megakaryocytes in the bone marrow (Walz et al. 2001).

Another important feature of BVDV infection in cattle is the ability of the virus to cause immunosuppression in acutely infected cattle. Decreases in the number of circulating immune cells, particularly B and T lymphocytes, lymphoid depletion in lymph nodes and Peyer's patches, and decreased function of cells from innate and adaptive immune response components have been reported (Ellis et al. 1988; Welsh et al. 1995; Walz et al 2001). Leukopenia is common in acutely infected animals and is usually characterized by neutropenia and lymphopenia (T and B lymphocytes) (Ellis et al. 1988). The result of immunosuppression in acute BVDV infections is an increased susceptibility to polymicrobial infections, particularly with viruses such as bovine herpesvirus type 1 (BHV-1), and bacteria such as Mannheimia hemolytica and Mycoplasma bovis (Ridpath 2010). Infection with BVDV has also been reported to enhance other infectious

conditions such as enteritis caused by Salmonella spp., colibacillosis, metritis, and mastitis (Ames 1987).

Infection of reproductive tract tissue with BVDV has been associated with decreased fertility and decreased conception rates in female cattle (Houe et al. 1993). Reduced conception rates in nonpregnant cows after acute BVDV infection have been related to infection and inflammation of the ovaries, reduced follicular growth, and decreased hormone production (Grooms et al. 1998a). Both persistently and acutely infected bulls shed BVDV in semen, and the virus is transmitted to susceptible cows by natural breeding or artificial insemination (Paton et al. 1990; Kirkland et al. 1991). A combination of factors, including lower quality semen, ill-thrift of affected bulls, and effects of BVDV on the reproductive tract of exposed cows are responsible for lower conception rates when using semen from bulls acutely or persistently infected with BVDV (Grooms 2004). After acute BVDV infections in bulls, the virus may reside in the testes following transient infection and viremia. This phenomenon has been detected after natural and experimental infections and is referred to as prolonged testicular infection (Givens et al. 2009). Localized, prolonged testicular infections with BVDV have been experimentally produced following acute infection of peri-pubertal bulls with BVDV (Givens et al. 2003; Givens et al. 2007). Viral RNA was detected in semen for 2.75 years following BVDV exposure, and infectious BVDV was isolated from testicular tissue for up to 12.5 months after BVDV exposure (Givens et al. 2009).

*Infection of the developing fetus*

Infection of the dam with BVDV during gestation may be clinical or subclinical since the majority of acute BVDV infections in immunocompetent cattle present only moderate clinical signs (Ames et al. 1986); however, infection of the fetus through the transplacental route appears to be highly efficient with BVDV (Dufell et al. 1985). The outcome of fetal infection with BVDV will depend on the biotype, the virulence of the virus and the gestational age of the fetus. In general, transplacental infection with BVDV may result in early embryonic death, abortion, mummification, congenital defects, stillbirths, normal calves born seropositive to BVDV, and persistently infected calves immunotolerant to BVDV (Grooms 2004).

Early embryonic death after BVDV infection in pregnant cows is an important cause of reduced reproductive performance in cattle herds (McGowan et al. 1995). Studies have confirmed that transplacental infection with BVDV during the pre-implantation period (29 to 41 days of gestation) results in considerable pregnancy losses in cattle (Carlsson et al. 1989, Grooms 2004). In contrast to dramatic pregnancy losses associated with transplacental infections with BVDV before day 41 of gestation, fetuses that survive infections with noncytopathic strains of BVDV during 45 to 125 days of gestation, develop persistent infection and immunotolerance to that specific BVDV strain (Grooms 2004).

Abortions caused by BVDV infections are usually concentrated during the first trimester of gestation between 50 and 100 days (Grooms 2004); however, although rare, mid and late term abortions and stillbirths have been reported after BVDV infection in cattle (Grooms 2004). Early fetal death can result in mummification, and expulsion of the fetus may take longer than 50 days after BVDV exposure (McGowan et al. 1995). Abortions

and reproductive failure are of critical importance in the economic losses associated with BVDV infections in cattle herds (Grooms 2004).

*In utero* infections with BVDV in pregnant cattle during the organogenesis period (100 - 150 days of gestation) can result in congenital malformations in newborn calves (Grooms 2004). Congenital abnormalities associated with BVDV infection appear to be frequently associated with defects in the development of the central nervous system and include cerebellar hypoplasia, hydrocephalus, hydrancephaly, optic neuritis, and microencephaly. Other congenital abnormalities include cataracts, retinal atrophy, brachygnathism, thymic aplasia, hypotrichosis, pulmonary hypoplasia, and growth retardation (Grooms 2004). Fetuses that are infected with BVDV after development of immunocompetence (100 – 125 days of gestation) may appear normal at birth and are seropositive to BVDV (Dufell et al. 1985). Calves with precolostral serum antibody titers against BVDV have a higher risk of becoming severely ill during the first month of life, indicating that although they were able to survive BVDV infection during late gestation, they are susceptible to developing disease early in life (Munoz-Zanzi et al. 2003).

*Infection of immunotolerant cattle (persistently infected animals)*

Persistently infected (PI) calves are the result of an *in utero* infection with noncytopathic BVDV during 45 to 125 days of gestation which corresponds to the gestational age before the development of immunocompetence (Grooms 2004). Immunotolerance of persistently infected animals is specific for the infecting noncytopathic strain of BVDV, and PI animals are able to respond immunologically and become seropositive to heterologous BVDV strains (Collen et al. 2000). The majority of

PI calves are born weak, they usually fail to grow equivalent to their cohorts, and 50% of them may die during the first year of life (Houe et al. 1993); however, some PI animals are born without abnormalities and are impossible to distinguish from normal healthy cattle (Baker 1995).

Persistently infected cattle usually have impaired immune responses and are more susceptible to opportunistic pathogens than their cohorts. This may be the reason why high mortality rates are observed in PI calves during the first year of life (Houe et al. 1995). Additionally, PI cattle are at risk of developing severe mucosal disease when they become "superinfected" with a cytopathic BVDV strain highly homologous to the noncytopathic BVDV strain responsible for the persistent infection (Bolin et al. 1985; Bolin 1995). Acute, chronic, and delayed onset mucosal diseases have been described (Bolin 1995). The severity and clinical features of each form of mucosal disease will depend on the degree of antigenic homology between the persistently infecting noncytopathic isolate and the superinfecting cytopathic isolate (Bolin 1995).

Acute mucosal disease develops when superinfecting cytopathic BVDV shares close antigenic homology with the PI noncytopathic BVDV strain and is considered fatal (Bolin 1995). Studies have suggested that acute mucosal disease results from genetic rearrangement and mutation of the persistently infecting noncytopathic BVDV strain and a *de novo* generation of a highly homologous cytopathic BVDV strain (Tautz et al. 1994). Clinical manifestations of acute mucosal disease include high fever, depression, fibrino-hemorrhagic diarrhea, mucopurulent oculonasal discharge, and erosions and ulcers in the gastrointestinal tract, coronary band, prepuce, and vulva (Evermann et al. 2005).

Chronic mucosal disease results from superinfection with a cytopathic BVDV strain that is heterologous with respect to the PI noncytopathic BVDV strain. Although chronic mucosal disease might not be fatal, chronic clinical signs are observed (Baker 1995). The source of the heterologous strain is usually external and does not involve mutation or genetic rearrangement of the noncytopathic isolate (Bolin 1995). Clinical manifestations of chronic mucosal disease include diarrhea, weight loss, chronic bloat, lameness, and erosions on epithelial surfaces (Baker 1995). Recovery from clinical signs can be observed if the degree of heterology between strains is enough to induce an adequate immune response to clear the heterologous cytopathic BVDV infection; however, persistent infection with the original noncytopathic strain will still remain (Bolin 1995).

Delayed onset mucosal disease occurs when clinical manifestations of acute mucosal disease are observed several weeks or months after inoculation with a heterologous cytopathic BVDV isolate (Westenbrink et al. 1989). The source of the heterologous strain is external and has been associated with modified live viral vaccines (Ridpath et al. 1995). Some studies have suggested that recombination occurs between RNA from heterologous cytopathic BVDV and RNA from the PI noncytopathic BVDV, resulting in the creation of a new cytopathic BVDV isolate identical to the resident noncytopathic BVDV (Ridpath et al. 1995). Clinical signs are similar to those seen in acute mucosal disease.

*Transmission*

Several direct and indirect routes of transmission have been described for BVDV within and between cattle herds (Thurmond 2005); however, the presence of persistently infected (PI) animals is considered the principal reservoir of the virus and the main source of BVDV transmission in cattle populations (Houe 1999). Direct contact of susceptible cattle with PI animals results in higher BVDV transmission rates compared with direct contact with acutely infected cattle (Thurmond 2005). Lower amounts and shorter duration of viral shedding have been described in cattle acutely infected with BVDV compared to PI cattle (Dufell et al. 1985, Niskanen et al. 2000). Virus concentrations are high in oculonasal secretions, saliva, feces, urine, uterine discharges, semen, and milk from PI cattle (Houe 1995; Lindberg et al. 2004; Thurmond 2005).Therefore, shedding of BVDV in natural secretions from PI cattle results in constant contamination of the environment and increased transmission rates to susceptible naïve cattle (Houe 1999; Thurmond 2005). Passively acquired BVDV-specific antibodies from maternal colostrum provide protection against acute BVDV infection. In PI calves born from acutely infected dams, colostral antibodies reduce viremia and viral shedding, thus diminishing the amount and duration of infectiousness and affecting transmission (Thurmond 2005). Additionally, the presence of maternally-derived BVDV-specific antibodies in serum from PI calves decrease viremia and isolation of the virus in these animals (Brock et al. 1998; Thurmond 2005).

Rapid BVDV transmission occurs when PI animals are born or introduced into the herd (Houe 1999). In one study, all susceptible cattle that came in contact with a PI animal in the herd became seropositive in less than 3 months (Moerman et al. 1993). The purchase of a PI animal or of a pregnant heifer carrying a PI fetus represents the highest risk of

introduction of BVDV into susceptible herds (Houe 1999; Van Campen 2010). Management practices of intensive cattle operations aid in the maintenance and dispersal of BVDV by purchasing pregnant heifers from facilities that commingle many cattle from different origins (Van Campen 2010). The risk of introducing a PI animal into the herd (including a PI fetus from a pregnant heifer) when buying 20 cattle of unknown origin and without testing is estimated to be 33% (Houe 1999). Introduction of BVDV to naïve herds through the purchase of acutely infected cattle can also result in BVDV transmission; however, factors such as virulence of the BVDV strain and severity of clinical signs may affect the dynamics of viral spread and transmission (Houe 1999). The risk of introduction of an acutely infected animal when buying 20 cattle is estimated to be 8% (Houe 1999). Purchase of animals without testing or quarantine is a critical route of introduction of BVDV into susceptible herds (Thurmond 2005).

Contact with cattle from other herds while pasturing or in shows may also be an important route of introduction of BVDV into susceptible herds. Pasturing of cattle at a distance of less than 5 meters to cattle from another herd was moderately associated with BVDV seroconversion; however, cattle in these herds were not tested for presence of PI animals (Houe 1999). Housing PI animals within 1 to 10 meters in proximity with susceptible cattle resulted in BVDV infection (Niskanen et al. 2003).

Adequate biosecurity measures are a key management factor in the prevention of introduction of BVDV into susceptible herds as well as in the transmission of the virus within herds (Ezanno et al. 2008). All purchased cattle and their offspring should be isolated and tested for PI status before entry into the herd, semen only from test negative bulls should be used, animals leaving the herd for shows should be quarantined for 3

weeks before re-entry, and young stock should be separated from adults (Ezanno et al. 2008). Additionally, several studies emphasize the importance of improving herd immunity through vaccination and testing and removal of PI animals early in life to effectively clear BVDV infections from cattle herds (Cherry et al. 1998; Smith et al. 2010).

While persistently infected (PI) animals are the major source of virus transmission within and between cattle herds, transmission of BVDV in the absence of PI animals has been described (Moerman et al. 1993; Moen et al. 2005), indicating that other transmission routes are important in the epidemiology of the disease (Houe 1999). The birth of non-surviving PI fetuses, fence to fence contact with infected cattle from other herds, contact with heterologous infected domestic livestock, contact with wildlife, use of contaminated semen or vaccines, use of contaminated fomites, airborne transmission, and hematophagous insects have been suggested as alternative routes of BVDV transmission within and between herds in absence of PI animals (Tarry et al. 1991; Gunn 1993; Houe 1999; Niskanen et al. 2002; Niskanen et al. 2003; Moen et al. 2005).

Bovine viral diarrhea virus can persist in tissues such as testes, ovaries, and white blood cells from acutely infected animals that have mounted an adequate and neutralizing immune response after the acute BVDV infection. Viral persistence in immune-privileged sites could contribute to BVDV transmission in cattle herds where the presence of PI animals has been determined to be absent (Houe 1999; Grooms et al. 1998b; Givens et al. 2003; Collins et al. 2009; Givens et al. 2009). Bulls acutely infected with BVDV were able to shed the virus in semen for 7 months after experimental induction of acute infection (Givens et al. 2003). Additionally, experimental inoculation

of BVDV-naïve calves with semen samples from these bulls 5 months after induction of acute infection resulted in viremia and seroconversion in the calves (Givens et al. 2003). Experimental transmission of BVDV to susceptible cattle through semen from acutely infected bulls has been demonstrated under field conditions (Kirkland et al. 1997); however, the potential of BVDV transmission from bulls with prolonged testicular infections is not clear and appears to be low (Givens et al. 2009). Bovine viral diarrhea virus antigen has been detected in ovarian tissues up to 60 days after inoculation in cows (Grooms et al. 1998a). Whole blood from BVDV seropositive animals previously inoculated with BVDV 98 days earlier was transfused to BVDV naïve cattle which developed viremia and seroconversion after transfusion. Bovine viral diarrhea virus RNA was intermittently detected in white blood cells from initially seropositive calves (Collins et al. 2009).

Infections with BVDV are not limited to cattle but can also be found in many other species from the mammalian order Artiodactyla. Infections with BVDV in heterologous species may play a critical role in the epidemiology of BVDV in cattle (Passler et al. 2010). Epidemiological and experimental evidence exists for BVDV infections in diverse species such as sheep, goats, pigs, camelids, and deer (Passler et al. 2010). Persistently infected heterologous species raise concern about the implications of non-bovine species as a potential route of transmission of BVDV to susceptible cattle (Passler et al. 2010). Indirect transmission of BVDV through contact with infected surfaces, embryos, fomites, vaccines, and flies has also been reported (Houe 1999; Thurmond 2005). Survival and infectivity of BVDV in the environment is short, and it is unlikely that the virus persists under dry conditions beyond 7 to 14 days (Baker 1987; Houe 1995). Translocation of

BVDV naïve cattle to unclean, non-disinfected areas that were previously occupied by PI animals may result in BVDV transmission (Niskanen et al. 2003; Lindberg et al. 2004). Airborne transmission of BVDV was demonstrated when BVDV naïve calves were housed at 1.5 and 10 meters of distance from PI animals (Niskanen et al. 2003). Transfer of embryos from or to PI or acutely infected cows with BVDV is an effective route of introduction of BVDV into susceptible cattle herds (Thurmond 2005). Use of contaminated fetal calf serum for washing procedures is also an effective route of BVDV transmission through embryo transfer (Houe 1999). Although it has been suggested that appropriate embryo washing eliminates the risk of BVDV contamination, one study demonstrated that recommended washing procedures were ineffective for removal of BVDV from an in vitro fertilization system (Houe 1999).

Several fomite vehicles such as hypodermic needles, nose tongs, rectal gloves, and the use of modified live or contaminated vaccines have been demonstrated to be associated with BVDV transmission within cattle populations (Gunn 1993; Houe 1999; Lindberg et al. 2004).

Although further research is necessary to determine their epidemiologic importance, insects such as stable flies, horse flies, and face flies can potentially serve as a source of BVDV infection to naïve cattle (Gunn 1993; Tarry et al. 1991). Horse flies (Hematopota pluvialis) and stable flies (Stomoxys calcitrans) were able to transmit BVDV to susceptible cattle after feeding on a persistently infected steer (Tarry et al. 1991). The virus was isolated from susceptible cattle from 72 hours to 10 days after the flies had fed on them, and BVDV was recovered from the flies (H. pluvialis and S. calcitrans) 96 hours after feeding (Tarry et al. 1991). Seroconversion was demonstrated in cattle

exposed to H. pluvialis previously infected with BVDV (Tarry et al. 1991). In another study, BVDV was isolated from face flies (Musca autumnalis) that had fed on a PI bullock; however, experimental transmission with the face flies was not attempted (Gunn 1993). A recent study experimentally evaluated the ability of the horn fly (hematobia irritans) to transmit BVDV from PI cattle to naïve calves. Flies collected from PI cattle tested positive to BVDV by RT-PCR and VI; however, transmission of BVDV did not occur after letting horn flies collected from PI cattle feed on BVDV naïve calves for 48 hours. The calves remained seronegative to BVDV and the virus was not detected by RT-PCR and VI during the time of the study (Chamorro et al. 2011). To the author's knowledge, evaluation of other insects such as mosquitoes, midges, lice, and ticks, as potential routes for BVDV transmission has not been performed.

*Diagnosis*

Due to the diverse clinical and subclinical syndromes displayed by animals infected with BVDV, a definitive diagnosis of infection is only possible through laboratory testing. Several factors including the management system of the affected farm, financial constraints, and availability of tests at a given laboratory will influence the selection of the appropriate method of BVDV testing (Walz et al. 2010). In order to obtain a successful result when selecting a diagnostic test for BVDV infection, a thorough evaluation of the clinical syndrome that is being diagnosed should be made to avoid selection of inappropriate tests (Saliki and Dubovi 2004). The most important clinical syndrome to diagnose BVDV in a herd situation is the identification and elimination of PI animals from the herd to prevent virus shedding and spread among

other cattle; however, not all tests are appropriate for identification of PI animals. Diagnostic tests for BVDV can be classified into one of four main categories: virus isolation; detection of BVDV antigen; reverse-transcription polymerase chain reaction (RT-PCR), and serology.

*Virus isolation.* Isolating virus from tissues or body fluids of infected animals remains the "gold standard" diagnostic test for BVDV (Saliki and Dubovi 2004; Edmondson et al., 2007;Walz et al., 2010). Different types of cells can be used for BVDV growing in cell cultures; however, cell lines used in BVDV isolation assays are usually limited to bovine turbinate, bovine testicle, and Madin Darby Bovine Kidney (MDBK) cells (Saliki and Dubovi 2004). The infection with cytopathic strains of BVDV results in the characteristic cytopathic effect in cultured monolayers of cells; cells demonstrate vacuolization and apoptosis following 48 hours of BVDV infection. The characteristic cytopathic effect is not demonstrated in monolayers of cells infected with non-cytopathic strains of BVDV which are the most common strains found in the field. Consequently, alternative visualization techniques such as immunofluorescence or immunostaining must be employed to detect infected cells.

The preferred sample to detect BVDV in live animals by virus isolation are white blood cells extracted from whole blood (Saliki and Dubovi 2004). Serum samples are also commonly used although false negative results may be obtained as a result of the presence of serum-neutralizing antibodies. One study reported that BVDV virus isolation performed on serum resulted in the lowest consistency in detecting positive samples and the lowest level of agreement between laboratories (Edmondson et al., 2007). Nasal swab samples are important samples to detect BVDV by virus isolation in experimental studies

where cattle has been challenged with BVDV. Detection of BVDV in nasal swab samples in this case demonstrate viral shedding. Tissue from the lymphoid organs such as thymus, spleen, Peyer's patches and mesenteric lymph nodes is the preferred sample to be collected from a dead animal or aborted fetus for submission for virus isolation (Saliki and Dubovi 2004). Persistent and acute infections cannot be differentiated with a single virus isolation test. To detect PI animals by virus isolation serial samples collected at 14-day intervals need to be collected and submitted for diagnosis (Brock 1995).

*Detection of BVDV antigen.* Although detection of direct antigen is generally less sensitive and less reliable than virus isolation techniques, antigen detection assays are generally quicker and more economical to perform than virus isolation assays (Saliki and Dubovi 2004). For this reason antigen detection methods are the most common diagnostic methods used to detect persistent infection with BVDV. Antigen detection methods include antigen-capture ELISA (ACE) and immunohistochemistry (IHC) techniques. Antigen detection tests are generally used as screening tests to detect PI animals although additional testing may be necessary in certain situations due to the presentation of false-positive results (Saliki and Dubovi 2004;Fulton et al., 2005). Immunohistochemistry techniques are usually performed by trained personnel at diagnostic laboratories. In contrast, commercially available ACE kits have been used cow-side for the identification of PI animals. Most commercial IHC testing is performed on fresh or formalin-fixed tissue (skin) samples commonly taken from the ear which care commonly known as "ear notches". However, differences in test sensitivity were not found when using tail fold biopsies or nasal swab specimens as test samples in one study (VanderLey et al., 2011). Other studies have reported a high level of sensitivity for the

commercial ACE kits when used as a screening test to detect PI animals (Fulton et al., 2005; Kuhne et al., 2005; Edmondson et al., 2007). Commercial ACE kits use monoclonal antibodies specific for the $E^{rns}$ glycoprotein of BVDV; therefore, differentiating strains of BVDV is not possible by the test (Gripshover et al., 2007).

*Reverse-transcription polymerase chain reaction.* Detection of viral RNA has become a common technique to detect BVDV infection in experimental and field situations (Driskell and Ridpath 2006). The amplification of the RNA genome by reverse-transcription polymerase chain reaction (RT-PCR) and subsequent detection is nowadays widely used as a BVDV diagnostic method. The technique is highly sensitive and can be utilized for various diagnostic samples including milk, urine, tissues, serum, whole blood, semen, and embryos (Hamel et al. 1995; Drew et al. 1999; Givens et al. 2001; Kim et al. 2003; Edmondson et al., 2007;Fulton et al., 2009). The RT-PCR assay should be validated for each sample type as poor RNA extraction will dramatically affect the sensitivity of the assay and its sensitivity depends not only on primer sets but also on the RNA extraction procedure (Saliki and Dubovi 2004). Although RT-PCR techniques can be used on diagnostic specimens that are fixed in formalin, RNA fragmentation could occur and reduce the assay sensitivity (Finke et al. 1993). Detection of viral RNA does not necessarily mean that infectious virus is present in the sample; therefore positive samples should be interpreted with care by clinicians evaluating individual or herd situations.

The high sensitivity of the RT-PCR assay allows the test to detect 1 positive BVDV serum sample in a pool of 100 negative serum samples (Kennedy et al. 2006). For this reason RT-PCR has become an attractive diagnostic tool for screening whole herds

or other groups of animals for the presence of BVDV by testing pooled milk or serum samples in an effective and economical way (Kennedy et al. 2006). The sensitivity of RT-PCR when using milk samples exceeds that of virus isolation nearly 15-fold (Radwan et al., 1995). However, it has been reported that the sensitivity of the test decreases when the number of samples included in the pooled aliquot increases (Ridpath 2011). It is important to mention that when using pooled tissue, whole blood, and serum samples for BVDV diagnosis, each animal in the herd must be sampled. In the case of bulk-tank milk samples, the BVDV infection status of any non-lactating animals (e.g., bulls, dry cows, heifers) on the farm will not be assessed. Recently, a screening diagnostic test for BVDV using RT-PCR that does not require individual sampling has been described for non-lactating animals (Givens et al., 2011). In this case, swabbing consumption surfaces and assaying the swabs by RT-PCR the investigators were able to determine the presence of PI animals in the herd.

*Serology.* The level of antibody response to previous exposure or challenge with a particular infectious agent can be used to demonstrate BVDV infection. The detection of serum antibodies that are directed against BVDV can be performed by various assays but the ELISA and serum neutralization tests predominate and have replaced indirect immunoperoxidase or indirect immunofluorescence techniques (Muvavarirwa et al. 1995). With appropriate application, serological tests allow the assessment of vaccine efficacy and compliance with established vaccination protocols, the detection of herd exposures to BVDV, and the association of clinical signs with BVDV infection. However, the interpretation of the results of serologic tests must be performed with care as these are unable to differentiate antibodies produced in response to natural exposure,

vaccination, or maternally derived through passive transfer from colostrum (Walz et al., 2010). However, in the absence of vaccination and when maternally-derived immunity has already decayed, serologic tests offer a good approximation to assess the herd's exposure status to BVDV (Saliki and Dubovi 2004). In vaccinated herds, testing of seronegative sentinel animals or performing paired samples to evaluate seroconversion (4-fold increase in initial antibody titer) may be useful to determine herd exposure. The serum neutralization assay is the most commonly used serologic test for BVDV infection and can be used to differentiate exposure to BVDV1 and BVDV2. However, comparison of results between different laboratories is complicated as different reference strains are likely used to determine the antibody titer (Walz et al., 2010).

## Prevention and control of bovine viral diarrhea virus infections

### *Introduction*

Identifying and eliminating BVDV reservoirs and reducing contact of susceptible animals with BVDV are the most important measures to prevent BVDV infections. Cattle persistently infected (PI) with BVDV are the most important source for BVDV transmission within cattle populations (Houe 1999). Acutely infected cattle are also an important source of BVDV; however, the rates of transmission are lower compared with contact with PI cattle (Thurmond 2005). Therefore control programs for BVDV should target 3 principal aspects: 1) Identification and elimination of PI cattle, 2) Implementing biosecurity measures to prevent contact of susceptible cattle with BVDV, and 3) Enhancing herd immunity to BVDV through vaccination (Brock 2003; Walz et al. 2010).

*Identification and elimination of persistently infected cattle*

Prevention of the entry of PI cattle into breeding dairy or beef herds should be the main goal of BVDV control programs. To adequately identify PI cattle, testing of every animal in the herd including calves, replacement heifers, pregnant cows, non-pregnant cows, and bulls should be performed (Larson et al. 2005). Pregnant cows that are negative for PI at testing time should be segregated before and after parturition and until a negative BVDV PI test is confirmed in the newborn calf (Smith et.al 2004). Dams from calves that test positive to BVDV PI should be tested for PI status as PI cows will always produce PI calves; however, most of PI calves result from acute BVDV infection of their dams during gestation (Smith et al. 2004). Additionally, aborted fetuses and dead or sick cattle should be tested for PI status. Diagnostic tests that detect specific BVDV antigens are the preferred tests to evaluate for PI status in the herd. Reverse transcription – polymerase chain reaction (RT-PCR), antigen capture ELISA (ACE), and immunohistochemistry (IHC) in skin samples are the most common tests used to screen herds for the presence of PI animals (Edmondson et al. 2007). Acutely infected animals can occasionally test positive to IHC, therefore positive animals of high genetic value should be re-tested during the following 30 days with RT-PCR (Cornish et al. 2005).

High sensitivity tests such as RT-PCR can be used in pooled samples of serum, white blood cells, skin, and milk to screen herds for the presence of PI animals (Weinstock 2001); however, results can vary among different laboratories indicating that effects of sample handling and specific conditions at each laboratory could influence the outcome of the test. Positive pooled samples should be re-tested by virus isolation, ACE, IHC, or RT-PCR methods.

*Implementing biosecurity programs*

Prevention of re-introduction of BVDV into a herd that has successfully identified and eliminated all PI cattle is essential in control programs. All purchased cattle entering into the herd should be tested for PI status and quarantined for at least 3 weeks. This will prevent transmission of BVDV from acutely infected animals to susceptible animals in the herd. When pregnant cattle is purchased and bought into the herd without knowing the PI status of the fetus, in addition of testing the dam, PI status should be confirmed in the offspring after calving. Semen for artificial insemination programs should be tested for BVDV before using in the herd (Givens et al. 2009). Additionally in embryo transfer programs, all recipients must be tested for PI status prior embryo transfer (Gard et al. 2007). Exposure to other cattle, ruminants, and wildlife through fence lines and any other mean should be eliminated or reduced at maximum (Passler et al. 2010). Cattle going to exhibitions should be quarantined at least 3 weeks before re-entry into the herd. Sanitation of equipment and people entering the farm could also reduce the risk of re-introduction of BVDV into the herd.

*Enhancing herd immunity through vaccination*

Successful vaccination of cattle against BVDV should protect against clinical signs of acute BVDV infection, reduce systemic spread of the virus (viremia), and reduce virus shedding (Ridpath 2013). Additionally, an effective vaccination program should prevent infection of target cells of the reproductive and lymphatic systems to prevent occurrence of fetal infection and immunosuppression, respectively (Kelling, 2004). The principal outcome of vaccination is the adequate induction of humoral and cell mediated

immune responses that will provide overall protection against challenge with field strains of BVDV. Induction of neutralizing antibodies through vaccination results in inactivation of infective BVDV virions, which prevents systemic spread of the virus in target tissues reducing clinical signs of disease and fetal infection (Kelling et al. 2004). Activation of specific cell mediated responses to BVDV (CD4+, CD8+, and γ/δ T cells) results in elimination of infected cells that have the potential of releasing infectious virus and therefore prevents clinical signs and viremia (Howard et al. 1992; Rhodes et al. 1999; Endsley et al. 2004). The induction of adequate cell mediated immune responses is critical for the resolution of acute infection with noncytopathic BVDV strains (Rhodes et al.1999).

Two types of BVDV vaccines can be found. Modified-live virus (MLV) and killed or inactivated virus (KV) vaccines. Cytopathic and noncytopathic BVDV strains can be found in MLV and KV vaccines. For many years, most vaccines contained only BVDV-1 strains. However, due to antigenic diversity between type 1 and type 2 strains and increased number of cases of clinical disease and persistent infection with BVDV 2 in vaccinated animals, modified-live and inactivated vaccines containing both type 1 and type 2 BVDV have been developed and are commercially available (Kelling 2004). Modified live virus vaccines are capable of inducing humoral and cell mediated immune responses offering a more balanced protection against acute BVDV infections. Additionally, a prolonged duration of immunity is expected when MLV vaccines are used. Disadvantages of MLV vaccines include the potential to cause immunosuppression, clinical disease, abortion, and persistent infection in vaccinated animals. Killed vaccines do not carry the risk of causing immunosuppression nor clinical disease; however, their

ability to induce adequate memory immune responses is reduced thus requiring a booster between 21-28 days after the first dose of vaccine. Although some studies have indicated that KV are capable of inducing T cell mediated immunity, others have failed to demonstrate significant cell mediated responses after vaccination of calves with KV BVDV vaccines (Murphy and Chanock, 2001; Endsley et al. 2003; Platt et al. 2008).

Protection against clinical disease and protection of the fetus through vaccination with MLV and KV BVDV vaccines have been demonstrated by several studies (Bolin and Ridpath 1989; Zimmerman et al. 2006; Givens et al. 2012); however, efficacy of vaccination against BVDV can be influenced by several factors such as: immune responses (humoral and cell mediated) induced after vaccination, fetal protection, crossreactivity among BVDV strains, duration of immunity, effect of maternal antibody on immune responses, purity, and reversion to virulence (Kelling, 2004).

*Immune responses to vaccination*. Response to vaccination has been evaluated in cattle principally through vaccination-challenge studies that measure clinical protection and serological responses (Menanteau-Horta et al. 1985; Bolin et al. 1991). Recently, some studies have focused on the evaluation of cell mediated memory responses to vaccination and challenge and their role in clinical protection in absence of antibody responses (Endsley et al 2004; Platt et al. 2009). Studies where challenge with virulent virus occurs after vaccination are more significant since not only demonstrate immune responses but evaluate clinical protection exerted by vaccination. Efficacy of vaccination can be demonstrated by the ability of the vaccine to clinically protect vaccinated animals against viral challenge compared with a non-vaccinated control group. Based on current research, a single dose of a MLV BVDV vaccine induce production of high concentration

of virus-neutralizing antibodies and induce T and B cell mediated memory responses that protect calves at challenge with BVDV (Endsely et al. 2003, Platt et al 2009). In contrast, KV BVDV vaccines are able to induce adequate antibody responses and T cell mediated responses but they need a booster to induce adequate immune memory (Kelling 2004, Platt et al. 2008).

*Fetal protection.* Field and experimental studies have discussed the efficacy of vaccines on fetal protection from BVDV infection (Oirschot et al. 1999; Van Campen et al. 1998). In one experimental study, 10 of 12 dams vaccinated prebreeding with MLV BVDV type 1 vaccine and experimentally challenged around 75 days of gestation with ncp BVDV type 1, were protected against giving birth to PI calves (Cortese et al. 1998a). Other study, reported that heifers vaccinated with a ncp MLV BVDV-1 (strain WRL) vaccine 21 days before breeding, and challenged at 55 to 100 days of gestation by intravenous administration of BVDV type 1 (strain 7443) had a significantly lower proportion of calves of PI calves at calving. In that study, 92% of calves born to vaccinated heifers were not persistently infected with BVDV (Dean et al., 2003). Givens et al. (2012), reported that pregnant heifers vaccinated once with a MLV vaccine before breeding were protected against abortion after challenge-exposure with 3 steers PI with BVDV 1a, BVDV 1b, and BVDV 2, respectively. Additionally, the proportion of PI calves after calving was significantly lower in vaccinated vs. non-vaccinated heifers; however, other studies have reported that PI calves were born in herds in which dams were vaccinated (Kelling et al. 1990; Van Campen et al. 2000).

Protection offered by vaccination with KV BVDV vaccines is adequate but incomplete as and only partial protection has been reported (Ellsworth et al. 1993). In one study, cattle

were vaccinated prebreeding with an inactivated BVDV vaccine and experimentally challenged with BVDV between 80 and 90 days of gestation. Only 36% of the fetuses were protected in this study (Ellsworth et al. 1993). Another study used the combination of both an inactivated BVDV vaccine followed by a MLV BVDV vaccine in heifers. Animals were intranasally challenged four weeks after the second vaccination (between 30 and 120 days of gestation) with a mixture of type 1 and type 2 BVDV isolates (Fray et al. 2002). All nine vaccinated heifers delivered clinically healthy seronegative and BVDV-free calves. On the other hand all non-vaccinated control cattle developed viremia and their offspring were PI. Therefore, vaccination can provide significant fetal protection reducing the occurrence of fetal infection. However, several authors have concluded that vaccination is not 100% effective preventing fetal infections and that biosecurity and surveillance for identification and elimination of PI cattle are necessary (Fray et al. 2002; Fulton and Burge 2000; Fulton et al. 2003).

*Crossreactivity.* Antigenic variation is important among BVDV isolates circulating in cattle populations and crossreactivity of BVDV vaccines is critical when evaluating vaccine efficacy. Inactivated and MLV BVDV vaccines are capable to stimulate production of antibodies that crossreact with a range of antigenically diverse strains of BVDV isolates. In addition, protection against challenge with heterologous virulent BVDV isolates has also been demonstrated for both types of vaccines (Kelling 2004).

Previous studies have demonstrated crossreactivity of the antibody response induced by MLV BVDV vaccines using *in vitro* neutralization assays. Vaccinated cattle produced antibodies that neutralized 10 to 20 strains of BVDV 1 and BVDV 2 *in vitro* 3

weeks after vaccination (Bolin and Ridpath 1989; Cortese et al. 1998b). Experimental vaccination-challenge experiments have confirmed that MLV vaccines containing BVDV 1 have the ability to protect calves from experimental infection with virulent BVDV 2 (Dean and Leyh 1999; Ellis et al. 2001). Similar to MLV vaccines, inactivated BVDV vaccines also have the ability to produce crossreactive antibodies against heterologous BVDV 2 isolates *in vitro* and protect against experimental challenge with heterologous BVDV strains (Fulton and Burge 2000).

Recent studies revealed that type 1b is the predominant BVDV 1 subgenotype, representing more than 75% of field isolates of BVDV 1 in the United States (Fulton et al. 2003a; Fulton et al. 2002). However, nearly all commercially available BVDV 1 vaccines contain BVDV 1a strains. Recent studies have demonstrated that calves vaccinated subcutaneously, intradermally or intranasally with a single dose of a multivalent, MLV vaccine containing a BVDV 1a strain, and subsequently challenged with different BVDV 1b strains were protected from clinical disease caused by the BVDV 1b viruses, as indicated by significantly lower rectal temperatures, reduced viral shedding, and greater white blood cell counts than non-vaccinated control animals. The BVDV 1a vaccine elicited efficacious protection against each BVDV 1b challenge strain, with a duration of immunity of at least 6 months (Xue et al. 2011; Xue et al. 2010).

*Duration of immunity.* Duration of immunity after vaccination is critical in the evaluation of efficacy of BVDV vaccination programs (Kelling, 2004). It is believed that modified-live virus BVDV vaccines generally produce a longer immunity compared with inactivated vaccines because their ability to induce stronger B and T cell specific responses with higher production of neutralizing antibodies and enough cell mediated

memory response that results in fewer vaccine administrations (Kelling 2004). Immunization of BVDV-seronegative calves at 10–14 days of age with a single dose of a MLV BVDV 1 vaccine provided protection against development of severe disease when challenge with a virulent type 2 BVDV was performed 4.5 months after vaccination (Ellis et al. 2001). In another study, neutralizing antibody response was still detectable 18 months following vaccination of seronegative cows with MLV BVDV vaccine (Cortese et al., 1998b). In contrast, other studies have reported a more rapid decline of antibody titers. Decline in serum neutralizing antibodies was detected 4.7 months following vaccination of calves in one study (Fulton and Burge 2000). Other study reported that antibody titers declined at 3.5 months following the administration of a single dose of a MLV BVDV vaccine to seronegative calves; however, calves were protected to BVDV 2 challenge (Dean and Leyh, 1999).

*Effect of maternal antibodies on immune response.* Vaccination of calves in the face of maternal antibodies (IFOMA) against BVDV is challenging as maternally-derived immunity interfere with the activation of adequate antibody responses to vaccination. Vaccination of calves IFOMA generally does not result in seroconversion; however, it generally primes T and B cell memory responses that result in protection against clinical disease at viral challenge later in life when maternal immunity has disappeared (Ridpath et al. 2003; Platt et al. 2009). The magnitude and effectiveness of the immunologic responses induced by vaccination IFOMA against BVDV have been evaluated thoroughly (Ellis et.al 2001; Zimmerman et al. 2006; Platt et.al 2009). Immune responses to vaccination IFOMA against BVDV are variable among calves as it is the degree of clinical protection against challenge (Ellis et.al 2001; Zimmerman et al. 2006; Platt et.al

2009). Factors such as calf age, level of maternally-derived antibodies, type of vaccine, and route of administration play a critical role in the outcome of vaccination and in the level of protection against viral challenge (Brar et.al 1978; Menanteau-Horta et.al 1985; Ellis et.al 2001; Platt et.al 2009).The ultimate goal of vaccination of calves IFOMA is to prevent acute clinical disease and virus transmission when calves are exposed to field strains of BVDV.

*Purity.* Contamination of modified-live virus vaccines with adventitious pestiviruses, particularly virulent BVDV during the manufacturing process represents a significant risk factor for induction of acute BVDV disease (Barkema et al. 2001). Commercial vaccines contaminated with exogenous BVDV strains could cause severe clinical consequences in vaccinated cattle. In Europe, severe outbreaks of BVDV 2 infection with ≥70% morbidity have been associated with the use of batches of MLV bovine herpesvirus 1 (BoHV-1) marker vaccine contaminated with BVDV (Barkema et al. 2001; Falcone et al. 1999; Jagodzinski et al. 2002). The main source of adventitious BVDV is fetal bovine serum which is used as a supplement in cell culture medium to grow vaccine virus in cell cultures (Rossi et al. 1980). A BVDV contamination rate in bovine sera has been estimated to be between 10 and 75% (Bolin 1991; Rossi et al. 1980). Current U.S. licensed viral vaccines are unlikely to be contaminated with pestiviruses as demonstrated by a previous study (Audet et al. 2000). However, it is recommended that bovine sera and cell lines used for cell cultures are screened for adventitious BVDV contamination by using improved reagents (eg, monoclonal antibodies) or new technology (eg, RT-PCR) with higher sensitivity. Additionally, fetal

bovine serum that pass rigorous testing procedures and are negative for virus may be irradiated or chemically treated as a further preventive measure.

Xia et al. (2011) investigated 33 batches of fetal bovine serum (FBS) obtained from ten suppliers (from different countries) for the presence of both recognized and atypical bovine pestiviruses contamination. All 33 batches of FBS were positive by real-time RT-PCR assays for at least one species of bovine pestiviruses. According to the certificate of analysis that the suppliers claimed for each batch of FBS, BVDV 1 was detected in all countries and BVDV 2 was detected exclusively in America. Atypical pestiviruses were detected in 13 batches claimed to originate from five countries. This study demonstrated that commercial FBS batches of different geographic origins could be contaminated with bovine pestiviruses.

*Reversion to virulence.* There is potential risk for shedding of vaccine virus from animals vaccinated with MLV BVDV vaccines, transmission of BVDV to susceptible animals, and reversion of BVDV vaccine strain to virulence. Different reports suggest that calves vaccinated with MLV BVDV vaccines develop transient viremia (Fulton et al. 2003b; Hunsaker et al. 2002). Vaccine virus can be found in nasal secretions for several days following vaccination of calves (Hunsaker et al. 2002). In contrast, another study reported that BVDV from a MLV vaccine was not shed nor transmitted from vaccinated calves to control calves (Fulton et al. 2003b).

*Bovine viral diarrhea virus vaccination programs*

The main objective of a vaccination program in beef and dairy operations is to induce maximal protective responses in cattle when the risk of BVDV infection is at its

greatest during the production cycle. In beef and dairy herds the pre-weaning and pre-breeding stages represent the most critical times when immunity should be high in order to protect against reproductive losses, immunosuppression, and respiratory tract disease associated with acute BVDV infections (Kelling, 2004).

*Vaccination of replacement heifers and cows in beef operations.* Prevention and control of BVDV infections cannot rely only in vaccination to protect against fetal infection and acute clinical disease. Other measures such as identification and elimination of PI cattle and establishing biosecurity measures are important to guarantee the success of any BVDV vaccination program (Audet et al., 2000). Based on results from current research it is recommendable to use MLV BVDV vaccines to vaccinate replacement heifers and cows prior to breeding to provide maximal protection against fetal infection (Givens et al. 2012). Several field and experimental studies have recommended vaccination of unstressed, healthy heifers, separated from pregnant cows, MLV BVDV vaccines (Givens et al. 2012). Vaccination of all replacement heifers should be performed twice before breeding. It is recommended to begin this vaccination protocol three estrous cycles (ie, 2 months) before breeding. Cows should be revaccinated annually 2-4 weeks before breeding. If inactivated vaccines are used, administration of vaccine should be timed so that maximal responses are achieved at the time of breeding. It is also essential to follow the vaccine manufacturer's recommendation about the dose, timing of booster vaccinations, and number of boosters (Kelling, 2004).

*Vaccination of replacement heifers and cows in dairy operations.* Cows in a dairy herd are normally at various reproductive stages. This limits selection of vaccines to those that are safe to use in pregnant animals. As discussed for beef cow–calf herds,

similar BVDV vaccination strategies might be recommended for dairy herds; however, it is possible that the use of MLV BVDV vaccines in replacement heifers and KV BVDV vaccines in adult cows is the safest method to avoid potential negative consequences of usage of MLV vaccines (Kelling 2004). Healthy replacement heifers should be vaccinated twice with a MLV BVDV vaccine during 30-60 days prior to breeding. These animals need to be isolated from pregnant cows during and after vaccination. Vaccination should be scheduled so that maximal protection is achieved during the critical first 4 months of gestation to maximize the potential for adequate duration of immunity and enhance protection against fetal infection (Kelling, 2004).

If inactivated vaccines are administered, vaccination of heifers before breeding should be timed so that maximal responses are obtained by the first 4 months of gestation to maximize protection against fetal infection. Thus, the second booster should be given 2 weeks before breeding. Cows should be revaccinated annually, 2 weeks before breeding (Kelling, 2004).

*Vaccination of the beef calf.* It is a common management practice in cow–calf operations to vaccinate young calves in late spring when processed at branding time. At this time maternal antibody titers are still elevated; however, recent studies have demonstrated the ability of MLV and KV vaccines to prime B and T cell responses that result in an increased production of antibodies and in the establishment of cell mediated memory responses in calves vaccinated IFOMA (Fulton et al. 2004; Platt et al. 2009). In contrast one study failed to demonstrate protection when very young calves with high levels of maternally-derived BVDV antibodies were vaccinated at 10-14 days of age with a MLV BVDV 1 vaccine and severe clinical disease was observed at challenge with

BVDV 2 4.5 months following vaccination (Ellis et al., 2001). The authors attributed the failure of protection to the interference exerted by maternal antibodies; however, age at vaccination has been demonstrated to be a critical factor when vaccinating very young calves (< 2 weeks of age) IFOMA. Usually calves under 2 weeks of age fail to prime adequate B cell responses after vaccination (Ellis et al. 2001; Platt et al. 2009). Therefore it is recommendable not to vaccinate calves under 2 weeks of age when adequate transfer of maternal antibodies has occurred. If calves are vaccinated at an early age, they should be revaccinated a 2-4 months later when maternal antibody titers have declined (Kelling 2004; Woolums 2007).

Weaned beef calves (6-7 months of age) are normally seronegative or have very low titers of maternally-derived antibodies to BVDV (Kelling et al. 1990). The stress of weaning and other factors around weaning time such as transport and commingling might result in immunosuppression and exposure to viral pathogens in beef calves. Therefore the time around weaning is considered the most critical period for the presentation of bovine respiratory disease complex (BRDC) in beef calves. Beef calves should be vaccinated a few weeks before weaning with a MLV or KV BVDV vaccine so that they achieve maximal protection against BVDV at weaning and have some degree of protection against BRDC. The use of MLV BVDV vaccines in pre-weaned calves carries a risk if still nursing a pregnant cow due to fetopathogenic potential of live vaccine BVDV strains. An effective strategy for optimizing virus neutralizing antibody titers in a preconditioning program is the use of an inactivated vaccine given to calves nursing pregnant cows followed by a modified-live BVDV vaccine at weaning (Grooms and Coe, 2002).

*Vaccination of the dairy calf.* Vaccination programs in dairy calves are going to be influenced by the colostrum management program and the prevalence of failure of passive transfer of immunoglobulins (FPT) in the herd (Windeyer et al. 2012). Thus, titers of maternally-derived BVDV antibody could vary widely depending on the quality and quantity of the colostrum consumed, as well as the efficiency of intestinal absorption of immunoglobulins. A high percentage of neonatal dairy calves with FPT would suggest that early vaccination is necessary as calves with no maternally-derived immunity to BVDV respond to vaccination early in life (Ellis et al. 2001); In a recent field trial where calves were vaccinated with a multivalent MLV vaccine containing BVDV at 2, 5, or 2 and 5 weeks of age, significant differences in the presentation of BRDC during the pre-weaning period were not observed (Windeyer et al. 2012). In this study the incidence of FPT was only 11%. Calves with FPT should be vaccinated as early as possible after birth but in the absence of FPT vaccination could be delayed until 7-8 weeks of age to obtain maximal benefits in antibody and cell mediated responses to vaccination (Platt et al. 2009).

Vaccination of calves against common respiratory viruses in the face of maternally-derived antibodies (IFOMA)

## Abstract

Vaccination of calves in the face of maternal antibodies (IFOMA) is challenging as maternally-derived immunity interferes with the activation of adequate antibody responses to vaccination. Vaccination of calves IFOMA generally does not result in seroconversion; however, it primes T and B cell memory responses that result in protection against clinical disease at viral challenge later in life when maternal immunity has disappeared. The activation of B and T cell memory responses in calves vaccinated IFOMA varies and is affected by several factors including age, level of maternal antibodies, type of vaccine, and route of administration. These factors can affect the outcome of vaccination IFOMA by influencing adequate priming of humoral and cell mediated immune responses. Failure to adequately prime adaptive immune responses following vaccination IFOMA could result in lack of clinical protection and increased risk of viremia and virus shedding. Therefore vaccination of calves IFOMA should be performed at a time optimal for priming of B and T cell memory immune responses.

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## Introduction

Viral respiratory pathogens such as bovine viral diarrhea virus 1 and 2 (BVDV 1 and BVDV 2), bovine herpesvirus 1 (BoHV-1), bovine respiratory syncytial virus (BRSV), and parainfluenza 3 virus (PI3V) play a critical role in the pathogenesis of bovine respiratory disease complex (BRDC) (Van Donkersgoed et al. 1994; Martin et al. 1999; O'Connor et al. 2001). A positive correlation between high levels of serum virus-neutralizing antibodies derived from colostrum or vaccination and decreased incidence of BRDC have been reported in calves (Martin et al. 1999; Fulton et al. 2004; Moerman et al. 1994).Vaccination against BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V is considered a key management strategy to minimize mortality and economic losses associated with BRDC in weaned calves (Peters et al. 2004; Step et al. 2009); however, higher intensification and specialization of beef and dairy operations have resulted in an increased incidence of BRDC before weaning (Woolums et al. 2013b). Additionally, changing conditions in climate and the beef market have lead producers to adopt early weaning practices in beef calves (Rasby 2007). These factors have contributed to an increased need for vaccinating calves against BRDC pathogens earlier than conventionally, when maternally-derived immunity is still present. For long time it was

believed that the presence of maternally-derived immunity interfered with the induction of adequate immune responses to vaccination; however, recent studies indicate that priming humoral and cell mediated immune responses is possible when vaccinating calves IFOMA (Ellis et al. 1996; Endsley et al. 2004; Patel et al. 2004; Platt et al. 2009).

The magnitude and effectiveness of the immunologic responses induced by vaccination IFOMA are variable among calves as it does the degree of clinical protection against infection (Ellis et al. 2001; Patel et al. 2005; Platt et al. 2009; Ellis et al. 2010). Factors such as calf age, level of maternally-derived antibodies, type of vaccine, and route of administration play a critical role in the outcome of vaccination and in the level of protection against viral challenge (Brar et al. 1978; Menanteau-Horta et al. 1985; Ellis et al. 2001; Patel et al. 2004; Platt et al. 2009).The ultimate goal of vaccination of calves IFOMA is to prevent acute clinical disease and virus transmission when calves are exposed to field strains of respiratory viruses. This article reviews the literature on the subject of vaccination of calves IFOMA and follows a similar approach used by a previous article published in 2007 (Woolums 2007). The objective of this review is continue to provide evidence-based recommendations on the clinical application of vaccination of calves IFOMA.

*Transfer of virus-specific antibodies from colostrum*

Virus neutralizing antibodies to BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V transmitted through maternal colostrum protect neonatal calves against acute clinical disease during the first months of life (Ridpath et al. 2003; Patel et al. 2004; Peters et al. 2004). The higher the titer and longer the persistency of maternal antibodies, the higher

the interference of antibody responses induced by vaccination (Munoz-Zanzi et al. 2002; Fulton et al. 2004); however, when the titer of maternal antibodies is low, interference to vaccination is minimal (Ellis et al. 2001; Munoz-Zanzi et al. 2002). Therefore the duration of maternal antibodies to respiratory viruses such as BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V depends mostly on the initial titer absorbed from maternal colostrum (Kirkpatrick et al. 2001; Munoz-Zanzi et al. 2002). The titer range of maternal antibodies to respiratory viruses in calves is highly variable (Kirkpatrick et al. 2001; Fulton et al. 2004; Chamorro et al. 2014). In one study the initial mean range of maternal antibody titers to respiratory viruses in 60-day-old calves varied from 0 to 935 for BoHV-1, 16 to 16384 for BVDV 1a, 8 to 8192 for BVDV 1b, 0 to 8192 for BVDV 2, and 0 to 4096 for BRSV (Fulton et al. 2004). In a recent study, the coefficient of variation of initial ranges of viral antibody titers derived from maternal colostrum in a group of 2-day-old calves were 28.03% for BVDV 1, 37.4% for BVDV 2, 24.98% for BRSV, and 43.49% for BoHV-1 (Chamorro et al. 2014).

High variation in the level of maternally-derived immunity among calves could affect the uniformity of ages at which seronegative status is reached and therefore can affect the response to vaccination IFOMA. In one study the standard deviation (SD) of the mean time to reach seronegative status to BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V in calves that received maternal colostrum at birth varied from 37 to 116 days (Kirkpatrick et al. 2001). Another study reported an 18 to 53-day variation of the SD of the mean time to reach seronegative status to the same viruses in calves that received maternal colostrum or a colostrum replacer (Chamorro et al. 2014). These results indicate that differences in antibody response to vaccination IFOMA should be expected in as

some animals will have higher and others will have lower titers for specific respiratory viruses at a determined age of vaccination (Kirkpatrick et al. 2001, Munoz-Zanzi et al. 2002).

*Immunologic responses induced by vaccination of calves IFOMA*

Methods to evaluate immunologic responses to vaccination include antibody production and seroconversion (Kelling et al. 1990; Kirkpatrick et al. 2008). Seroconversion is a 4-fold or greater increase in serum antibody titers to a specific infectious agent after vaccination or infection and is considered one of the standard measures of vaccine efficacy in calves (Brar et al. 1978; Kelling et al. 1990). The absence of seroconversion following vaccination of young calves could be interpreted as vaccine failure; however, protection against acute clinical disease caused by BVDV and BRSV has been demonstrated in the absence of seroconversion in calves vaccinated IFOMA (van der Sluijs et al. 2010; Stevens et al. 2011). Vaccination IFOMA does not usually result in seroconversion but anamnestic antibody responses at a second dose of vaccine has been demonstrated to occur in calves (Brar et al. 1978; Menanteau-Horta et al. 1985). Additionally, an increased persistency (reduced rate of decay) of maternal antibodies and development of specific-lymphocyte proliferative responses have been reported in calves vaccinated IFOMA (Ellis et al. 1996; Fulton et al. 2004).

In previous studies 28 to 84-day-old calves vaccinated IFOMA with MLV and KV containing BVDV 1, BoHV-1, PI3V, and BRSV did not initially seroconvert to the first dose of vaccination; however, at a second dose of the same vaccine given between 32 to 112 days later, anamnestic antibody responses were observed (Menanteau-Horta et

al. 1985; Kaeberle et al. 1998). In a more recent report, a group of calves vaccinated with a multivalent MLV vaccine at 67 days of age had a low proportion of calves that seroconverted to BVDV 1, BVDV 2, BoHV-1, and BRSV; however, the rate of decay of maternally-derived antibodies was decreased in vaccinated calves and anamnestic antibody responses were observed at the second dose of vaccine given at 190 days of age (Kirkpatrick et al. 2008). In another study (Munoz-Zanzi et al. 2002), vaccination of calves at 15 (KV vaccine) and 45 (MLV vaccine) days influenced the mean time at which calves became seronegative to BVDV 1 and BVDV 2. Depending on the initial level of maternally-derived BVDV 1 and BVDV 2 antibodies absorbed from maternal colostrum, vaccinated calves reached seronegative status earlier or later in life. When calves had an initial BVDV 1 titer > 512 or a BVDV 2 titer of > 32 no effect of vaccination was observed; however, when BVDV 1 titers were < 512 and BVDV 2 titers were < 32 vaccination increased the time at which calves became seronegative to each virus suggesting antibody production or increased persistency of maternal antibodies. Another study reported a reduced rate of decay of maternal antibodies after vaccination of calves with a multivalent KV vaccine at 60 and 155 days of age. The rate of seroconversion after vaccination at 60 days was minimal; however, the mean half-life of specific maternal antibodies to BVDV 1, BVDV 2, BoHV-1, and PI3V was increased similarly suggesting a prolonged persistency of maternally-derived immunity or activation of antibody production (Fulton et al. 2004).

An additional study reported that maternally-derived antibodies to BVDV 1, BRSV, BoHV-1, and PI3V decayed similarly until 140 days of age in calves vaccinated parenterally with a multivalent MLV vaccine at 2 or 70 days of age; however, calves

vaccinated at 70 days of age had a higher mean BVDV 1 antibody levels between 140 – 217 days of age compared with calves vaccinated earlier (Woolums et al. 2013). This suggests that age at vaccination IFOMA could influence the induction of B cell memory responses as younger calves appear to have a decreased ability to induce antibody production when vaccinated IFOMA. In contrast, specific cell mediated immune responses to BoHV-1 and BRSV can be primed in 10-day-old calves (Ellis et al. 1996). The ability to seroconvert to vaccination IFOMA depends on the level of maternal antibodies at the time of vaccination. The level of maternal antibodies that interferes with seroconversion is not uniform and varies among viruses. In one study, 84-day old calves with a BVDV 1 titer of 35 seroconverted to vaccination with a MLV vaccine; in contrast calves with BoHV-1 titers < 16 did not seroconvert to vaccination (Menanteau-Horta et al. 1985). Another study reported that calves with a BVDV 1 titer between 8 and 16 seroconverted to vaccination with a multivalent MLV vaccine; however, BVDV 2 titers between 4 and 8 prevented seroconversion to BVDV 2 (Kirkpatrick et al. 2001). In the same study, none of the calves with maternal antibody titers to BRSV or PI3V seroconverted to vaccination. Fulton R. et al. (2004), reported that maternal antibody titers of 128 for BVDV 1a, 32 for BVDV 1b, 128 for BVDV 2, 32 for BRSV, and 20 for BoHV-1 interfered with seroconversion to vaccination with a multivalent KV vaccine. Downey et al. (2013), suggested that calves with maternally-derived BVDV 2 antibody titer of 8.24 or higher did not seroconvert to vaccination with a MLV vaccine around 130 days of age.

Intranasal vaccination has been reported as an effective method of vaccination IFOMA to overcome the interference exerted by maternal antibodies. A recent study

reported that calves vaccinated at 2 to 3 days of age with an intranasal multivalent MLV vaccine and a booster with the same vaccine 35 days later did not seroconvert to vaccination but had a short duration increase in the titers of specific IgA antibodies to BVDV and BoHV-1 in nasal secretions. In the same study, INF-γ was not detected in serum or nasal secretions of vaccinated calves (Hill et al. 2012). Another study reported no differences in IgA levels of nasal secretions nor in the level of induction of cell mediated immunity to BVDV 1, BRSV, and BoHV-1 in calves vaccinated intranasally with a multivalent MLV vaccine at 2 or 70 days of age when compared with unvaccinated calves (Woolums et al. 2013).

*Response in calves vaccinated IFOMA and subsequently challenged with live viruses*

Reduction of clinical signs of disease has been repeatedly reported in calves vaccinated IFOMA with KV and MLV vaccines and subsequently challenged with BVDV or BRSV (Ridpath et al. 2003; Patel et al. 2004; Zimmerman et al. 2006; Woolums 2007). The priming of specific T cell memory responses following vaccination has been suggested as the main source of clinical protection of challenged calves (Endsley et al. 2003; Platt et al. 2009; Stevens et al. 2011); however, protection provided by vaccination IFOMA should not only prevent clinical disease but also viral replication, viremia, and virus shedding (Thurmond et al. 2001; Peters et al. 2004). Some studies have failed to demonstrate complete clinical protection or significant reduction of viremia and nasal shedding in calves vaccinated IFOMA and subsequently challenged with BVDV or BRSV (Ellis et al. 2001; Ellis et al. 2010; Stevens et al. 2011). One of these reports suggested that failure to induce anamnestic antibody responses to vaccination and

subsequent challenge with BVDV could result in a higher proportion of calves with viremia and viral shedding (Stevens et al. 2011). In contrast, results of other studies indicate that antibody response after vaccination IFOMA and higher antibody titers at challenge with BVDV or BRSV could be associated with decreased viremia and viral shedding (Vangeel et al. 2007; Chamorro et al. 2013).

Most of the experimental research in protection of calves against viral challenge following vaccination IFOMA has been performed with BVDV. Vaccination of 5-week old calves IFOMA with a multivalent MLV vaccine containing BVDV and challenged 3.5 months later with BVDV 2 resulted in protection against clinical disease, prevention of viremia, and reduction of leukopenia (Zimmerman et al. 2006). Another study demonstrated that calves inoculated IFOMA with virulent BVDV 2 between 2 and 5 weeks of age were protected against clinical disease at 7 to 9 months of when a second challenge with BVDV 2 was performed (Ridpath et al. 2003). In both studies, calves vaccinated IFOMA had some degree of antibody response at challenge and viremia was prevented in all vaccinated animals. Endsley J. et al. (2003) reported that calves exposed to BVDV 2 at 2 to 5 weeks of age developed BVDV 2 specific CD4, CD8, and γ/δ T cells and developed anamnestic antibody responses at challenge with BVDV 2. In the same study, calves vaccinated at 7 weeks of age with a KV or multivalent MLV vaccine containing BVDV developed anamnestic antibody responses at second vaccination at 14 weeks of age; however, the KV vaccine failed to induce specific BVDV T cell responses in vaccinated calves.

Failure to provide clinical protection and reduction of viremia and virus shedding have also been reported in calves vaccinated IFOMA. In one study, 10 to 14 day-old

calves vaccinated IFOMA with a MLV vaccine containing BVDV 1 were not protected at challenge with BVDV 2 4.5 months after vaccination. Sixty six percent of vaccinated calves in this study had to be euthanized due to severity of clinical disease (Ellis et al. 2001). In the same study, 10 to 14 day-old calves deprived from BVDV antibodies and also vaccinated, seroconverted to vaccination and were protected against challenge. The proportion of calves with viremia was higher in calves vaccinated IFOMA (50%) compared with calves vaccinated in absence of BVDV maternal immunity (15%). Another study demonstrated that vaccination of calves IFOMA at 3 days of age with a multivalent non-adjuvanted MLV vaccine resulted in clinical protection and reduction of mortality at 7 to 9 months of age when calves were challenged with BVDV 2. In this study, 80% of calves vaccinated IFOMA became viremic (Stevens et al. 2011). A more recent study reported that calves from 1 to 2, 4 to 5, and 7 to 8 weeks of age vaccinated IFOMA with a multivalent MLV vaccine containing BVDV 1, BVDV 2, BRSV, BoHV-1 and PI3V developed BVDV-specific T cell mediated responses. At challenge with BVDV 2 12 weeks after vaccination, all calves were clinically protected; however, only calves vaccinated at 4 to 5 and 7 to 8 weeks developed anamnestic antibody responses to BVDV after challenge. None of the calves developed antibody or T cell mediated responses to BRSV, BoHV-1, or PI3V after vaccination (Platt et al. 2009).

Recently, informative research has been published on protection against challenge with BRSV in calves previously vaccinated IFOMA parenterally or intranasally (IN). In one study 2 week-old calves vaccinated IFOMA at 2 and 6 weeks with a KV BRSV vaccine demonstrated significantly less clinical signs of disease and reduced viral shedding after challenge at 10 weeks of age compared with controls. Although

anamnestic antibody responses were not observed, vaccinated calves demonstrated a slower rate of decay of maternal BRSV antibodies compared with controls (Patel et al. 2004). A third dose of vaccine given at 18 weeks of age induced anamnestic antibody responses at challenge. Another study reported that 2 week-old calves vaccinated IFOMA with a KV BRSV vaccine and challenged with BRSV 21 days after vaccination did not develop anamnestic antibody responses at challenge; however, vaccinated calves were protected against severe clinical disease and had increased BRSV-specific IFN-γ production in peripheral lymphocytes 1 week after challenge (van der Sluijs et al. 2010).

Intranasal (IN) vaccination of calves with MLV vaccines containing BRSV has produced mixed results. Woolums AR. et al. (2004), reported that 4 to 6 week-old calves vaccinated IFOMA with an IN MLV vaccine and challenged at 8 weeks of age had reduced clinical signs of disease following challenge; however, viremia, virus shedding, and pathologic lung lesions were not different among vaccinated and unvaccinated calves. In this study vaccinated calves did not induce anamnestic responses at challenge; however, IFN- γ production was increased in lymphocytes from lymphoid bronchial tissue of vaccinated calves. Another study reported that 3 to 8 day-old calves vaccinated IN with a MLV BRSV vaccine were not completely protected against challenge with BRSV at 4.5 months of age. In this study vaccinated calves demonstrated similar levels of viremia, virus shedding, and lung lesions compared with controls. Antibody responses in serum and nasal secretions were not different in vaccinated and unvaccinated calves (Ellis et al. 2010). In the same study, IN or subcutaneous (SC) vaccination of 3 to 8 day-old BRSV-seronegative calves challenged with BRSV 21 days after vaccination resulted in clinical protection. Significant antibody responses or production of IgA in the upper

respiratory tract were not detected among vaccinated calves. A similar study reported vaccination of 2 to 9 week-old calves with a single component IN MLV BRSV vaccine. Vaccinated calves were protected at challenge 8 days after vaccination and had increased concentrations of IgA and IFN-α in nasal secretions. Additionally, vaccinated calves developed anamnestic antibody responses following BRSV challenge when 2 doses of vaccine were administered (Ellis et al. 2007).

*Field trials of vaccination IFOMA and prevention of bovine respiratory disease*

The best method to evaluate the efficacy of vaccination of calves IFOMA against natural infection with viral respiratory pathogens is through well designed field trials. Unfortunately, the inability to control the occurrence of natural disease and other logistic complications under field conditions limit the publication of these type of studies. A previous study described the efficacy of vaccination of calves at 3 and 5 weeks of age with a MLV BRSV vaccine and a Mannheimia haemolytica leucoctoxin/histophilus somni bacterial extract to reduce a high incidence of BRDC in calves from a farm where BRSV and Mannheimia haemolytica had been previously isolated. The proportion of calves that was treated for BRDC as determined by the owner was higher in calves that did not receive any vaccine (34%) compared with calves that received both vaccines (15%) (Van Donkersgoed et al. 1994). In another study on a dairy heifer rearing operation, calves were vaccinated IFOMA at 15 days of age with a multivalent KV vaccine containing BVDV 1 and at 45 days of age with a multivalent MLV vaccine containing BVDV 1. Exposure to BVDV measured as proportion of calves that seroconverted to BVDV 1 after vaccination was reduced in vaccinated calves for 60 days.

Additionally, it was estimated that the vaccination program in this operation prevented 48% of BVDV 1 transmission among calves from 4 to 9 months of age (Thurmond et al. 2001). A recent trial where 2874 dairy calves were used (Windeyer et al. 2012), reported that vaccination of calves with a multivalent MLV vaccine at 21 days, 28 days, or both did not reduce presentation of BRDC during the first 3 months of life. The incidence of failure of passive transfer (FPT) of experimental calves was 11% based on a total serum protein value at 24 hours of life < 5 g/L. The overall incidence of treatment for BRDC was 22% the and overall mortality rate was 3.5%. Differences among vaccinated and unvaccinated calves were not detected; however, the median calf age for BRDC treatment was 30 days and 44% of the cases of BRDC occurred before completion of the vaccination protocol. This could have affected the analysis of the actual effect of vaccination IFOMA in this study.

*Clinical recommendations based on current research*

Experimental trials of evaluation of efficacy of vaccination IFOMA against common respiratory viruses in young calves indicate that immunological responses and clinical outcome following vaccination are variable and could be influenced by several factors; however, some clinical recommendations can be made in order to obtain the greatest benefit when establishing programs of vaccination of young calves in the presence of maternal immunity:

- The high variation in duration of maternally-derived antibody titers to BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V among calves suggests that a source of maternal colostrum

with more uniform levels of specific antibodies to respiratory viruses could allow more consistent protection and more reliable estimation of time (age) of when to vaccinate.

- A single dose of a multivalent MLV vaccine containing BVDV and BRSV administered parenterally or intranasally to calves that received maternal colostrum and are older than 2 weeks of age can effectively protect against viral challenge later in life when maternal antibodies have disappeared.
- A single dose of a KV vaccine containing BVDV and BRSV administered to calves that received maternal colostrum and are older than 2 weeks could prime some immune responses and offer protection at viral challenge; however, the administration of 2 doses 2-4 weeks apart is recommended.
- Parenteral or intranasal administration of KV or MLV vaccines containing BVDV, and BRSV to calves younger than 2 weeks with high levels of maternally-derived antibodies should be avoided as it might not result in complete clinical protection at viral challenge later in life.
- Parenteral or intranasal administration of MLV vaccines containing BVDV or BRSV to calves deprived from specific-immunity or FPT effectively primes B and T cell responses and offer complete protection to calves later in life when challenged with virulent viruses.

## Conclusions

Vaccination of young calves IFOMA against BVDV and BRSV has demonstrated to prime humoral and cell mediated immune memory responses that provide different degrees of protection against viral challenge. Factors such as age at vaccination, level of

maternally-derived immunity, route of administration, and type of vaccine could affect immune responses of calves vaccinated IFOMA. Vaccination of very young calves with high levels of maternally-derived immunity results in activation of specific T cell memory responses that provide protection against clinical disease; however, activation of B cell memory responses is usually absent. It is possible that the high level of maternal antibodies and other cytokines and growth factors transferred in colostrum blocks activation of naïve B cells. Additionally, it is possible that immaturity of adaptive immune response in the neonate (low levels of complement and the low number of naïve B cells) could negatively affect B cell activation (Reber et al. 2005; Firth et al. 2005). The lack of anamnestic antibody responses at viral challenge could result in higher proportion of calves with viremia and nasal shedding and increase the risk of viral transmission.

Prevention of clinical signs of disease and reduction of viremia and virus shedding are the most important outcomes of vaccination IFOMA. Current research on vaccination IFOMA against respiratory viruses suggests that calves that fail to induce antibody production after vaccination and anamnestic antibody responses at viral challenge might have a higher risk of developing viremia and viral shedding (Chamorro et al. 2013). Failure to prevent or reduce viral shedding at challenge could increase the risk of virus transmission and affect overall calf-herd health.

## Statement of Research Objectives

In order to better characterize the effect of maternally-derived immunity against BVDV and in the humoral responses to vaccination of young calves and the protective effects of antibody production against viremia and virus shedding after challenge-exposure to BVDV or to BVDV and BoHV-1 this research work focused on the following objectives:

The first objective was to evaluate the duration and decay of maternally-derived antibodies against BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V in calves that receive maternal colostrum or a colostrum replacement product at birth.

The second objective was to evaluate the ability of three different modified live virus (MLV) vaccines containing BVDV 1 and BVDV 2 to induce antibody responses to vaccination in calves with different levels of maternally-derived BVDV antibodies.

The third objective was to determine if there were differences among three different MLV vaccines containing BVDV 1 and BVDV 2 to prevent clinical disease, viremia, and BVDV shedding after intranasal challenge of calves with virulent BVDV 2.

The four objective was to evaluate the ability of four different MLV vaccines containing BVDV 1a, BVDV 1b, BVDV 2, and BoHV-1 to induce antibody responses to vaccination in calves with different levels of maternally-derived BVDV 1, BVDV 2, and BoHV-1 antibodies.

The fifth objective was to determine if there were differences among four different MLV vaccines containing BVDV 1a, BVDV 1b, BVDV 2, and BoHV-1 to prevent clinical disease, viremia, and nasal shedding of BVDV and BoHV-1 after simultaneous natural exposure of calves to cattle persistently infected with BVDV and calves acutely infected with BoHV-1.

Comparison of levels and duration of detection of BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V antibodies in calves fed maternal colostrum or a colostrum replacement product


## Abstract

Colostrum replacements provide passive immunity to neonatal calves; however, their ability to provide adequate levels of antibodies recognizing respiratory viruses has not been described. The objective of this study was to compare the serum levels of IgG at 2 days of age and the duration for detection of BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V antibodies in calves fed maternal colostrum (MC) or a colostrum replacement (CR) at birth. Forty newborn male Holstein calves were assigned to CR or MC groups. Group CR (n=20) received 2 packets of colostrum replacement (100 g of IgG per 470 g packet), while group MC (n=20) received 3.8 L of maternal colostrum. Serum samples for detection of IgG and virus-antibodies were collected from each calf at birth, at 2 and 7 days, and monthly until the calves became sero-negative. Calves in the MC group had greater IgG concentrations at 2 days of age. The AEA was greater in MC group although not significantly different. The CR group had greater BVDV neutralizing antibodies during the first 4 months. The levels of antibodies to BoHV-1, BRSV, and PI3V were similar between groups. The mean time to sero-negativity was similar for each virus between groups; however, greater variation was observed in antibody levels and in the

duration of detection of immunity in the MC group. The CR product provided calves with more uniform levels and duration of antibodies against BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V. Calves that received MC at birth became sero-negative earlier in life and in greater proportions than calves that received CR.

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## Introduction

Bovine respiratory disease (BRD) is one of the most common diseases affecting cattle in the United States and is an important cause of economic losses in cattle operations worldwide (Griffin 1997; Stokka 2010). Viral respiratory pathogens such as bovine viral diarrhea virus 1 and 2 (BVDV 1 and BVDV 2), bovine herpesvirus-1 (BoHV-1), bovine respiratory syncytial virus (BRSV), and parainfluenza-3 virus (PI3V) play an important role in the pathogenesis of BRD due to their ability to impair the integrity of the upper respiratory tract, cause immunosuppression, promote secondary bacterial infection, and cause acute clinical disease (Martin et al. 1999; O'Connor et al. 2001, Ellis et al. 2010). Antibodies against respiratory viruses transmitted through maternal colostrum protect calves against acute BRD (Moerman et al. 1994; Marin et al.

1999; Fulton et al. 2002), and calves with high serum antibody titers against BVDV, BoHV-1, BRSV and PI3V at arrival to the feedlot present lower risk of developing BRD as evidenced by lower morbidity rates and reduced frequency of treatments (Fulton et al. 2002; Fulton et al. 2011). As maternally-derived immunity decays, calves become susceptible to acute BVDV infection and disease (Ridpath et al. 2003). The duration of maternally-derived immunity against respiratory viruses in the calf is directly proportional to the level of antibody to BVDV, BoHV-1, BRSV, and PI3 ingested and absorbed from colostrum (Kirkpatrick et al. 2001; Munoz-Zanzi et al. 2002). The higher the levels of serum antibody absorbed from colostrum, the longer the duration of the maternally-derived immunity (Munoz-Zanzi et al. 2002). However, previous research shows that there is a large range of initial serum antibody titers in calves that receive maternal colostrum (Kirkpatrick et al. 2001; Fulton et al. 2004), and as a consequence, variability in the age at which calves become susceptible to acute BVDV, BoHV-1, BRSV, and PI3V infections (Munoz-Zanzi et al. 2002, Fulton et al. 2004).

Provision of high quality colostrum to neonatal calves in a timely manner is a key management practice in the prevention of failure of passive transfer of immunoglobulins (FPT); however, maternal colostrum may not be consistently available, and as an alternative, commercial colostrum replacement products may provide adequate passive immunity while reducing the risk of infection with colostrum-transmitted pathogens (Godden et al. 2009a, Godden et al. 2009b, Pithua et al. 2009). When using colostrum replacement products, a minimum of 100 g of IgG is recommended to achieve acceptable levels of serum immunoglobulins, and feeding a higher mass of IgG (150-200 g) increases passive immunity and more reliably prevents FPT in calves (Godden et al.

2009a, Godden et al. 2009b). The ability of colostrum replacement products to provide calves with adequate levels of antibodies against BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V and the duration of the colostrum replacement-derived immunity has not been described. The objective of this study was to compare the serum levels and duration of detection of BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V antibodies in neonatal calves that receive maternal colostrum or a colostrum replacement product at birth.

## Materials and Methods

### *2.1. Calves and experimental treatment*

Forty newborn male Holstein calves from 3 dairy farms local to Auburn, Alabama were used in this study. Pertinent management practices in two of the dairies included pre-breeding vaccination of heifers with a modified-live vaccine that included BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V viral antigens and vaccination of adult cows with a killed vaccine that included the same antigens. One dairy farm did not vaccinate cattle against BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V. Calves were removed from their dams immediately after birth and strategically assigned to colostrum replacement (CR) or maternal colostrum (MC) groups. All calves (n=16) born on the farm that did not vaccinate against BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V viral antigens were assigned to the CR group. The strategic assignment in this study was necessary to assure the presence of serum virus-antibody titers in calves from the non-vaccinated herd after colostrum intake. Group CR (n=20, 16 from non-vaccinated herd and 4 from vaccinated herds) received 2 packets of a colostrum replacement (100 g of IgG/packet) (Land O

Lakes® Colostrum Replacement, Land O' Lakes Animal Milk Products, Shoreview MN) reconstituted with 1.5 liters of water per packet, while group MC (n=20, all from vaccinated herds) received 3.8 L of fresh or frozen maternal colostrum. Colostrum replacement product was not reconstituted following label directions; instead a larger water volume, 1.5 liters were used to reconstitute each packet of the product so volumes of CR and MC given to the calves were the same. Maternal colostrum was evaluated by a colostrometer (BIOGENICS®, Mapleton OR). If the specific gravity of the maternal colostrum was > 1.055 the calf received maternal colostrum from the dam. If the specific gravity of maternal colostrum was < 1.055 the calf received stored frozen colostrum with a specific gravity measurement above this level from cows from the same herd. Maternal colostrum or colostrum replacement was administered in a single feeding within the first 2 hours of life by bottle or esophageal feeder if the calf did not take the bottle during the first 10 minutes. Seven calves from the CR group and 11 calves from the MC group were fed with an esophageal feeder. After colostrum feeding, calves from both groups were moved together to individual pens in a calf isolation barn located at the Animal Health Research facilities at Auburn University. The isolation barn provided biosecurity to prevent exposure to BVDV, BoHV-1, BRSV, and PI3-V during the trial. After 12 hours of life, calves were examined and fed 1.8 liters of a commercial milk replacer (Land' O Lakes Animal Milk Products, Shoreview, MN) twice daily until weaned between 6-8 weeks of age. A calf starter, hay, and free choice water were offered to all calves from 1 week of age until weaning. Once weaned, calves from both groups were moved together to biosecure paddocks located at the Animal Health Research facilities at Auburn University, where calves had access to free-choice hay, water and a calf-grower grain

mix. During the study period, all calves were evaluated once daily and were cared for under the guidelines of Auburn University Institutional Animal Care and Use Committee.

*2.2. Sample testing*

Pre-colostral blood samples were collected from each calf for antibody testing and BVDV virus isolation (VI) testing. Any calf with positive results for BVDV testing or evidence of serum antibodies to any of the viruses in pre-colostral samples was removed from the study. Additionally, total serum IgG concentrations were evaluated in pre-colostral serum samples by single radial immunodiffusion. A second measurement of serum IgG concentration was performed in all calves at 2 days of age by single radial immunodiffusion. Failure of passive transfer of immunoglobulins was defined as a total serum IgG concentration < 10 g/L at 2 days of age (Weaver et al. 2000). Maternal colostrum and colostrum replacement samples were evaluated for IgG concentrations and the apparent efficiency of absorption of IgG (**AEA**) was calculated in all calves using the following formula: [**AEA** = serum IgG at 2 days of age (g/L) **x** plasma volume in L (estimated at 9.9% of body weight) / total IgG intake (g)] (Chelack et al. 1993; Quigley et al. 2002). Post-colostral blood samples were collected in all calves at 2 and 7 days of age, and monthly for virus antibody testing. Monthly sampling of calves continued until the calves became sero-negative to each virus or until 8 months of age. Serum neutralizing antibody titers against BVDV 1 and BVDV 2 were measured through VN testing, while BoHV-1, BRSV, and PI3V antibodies were measured through indirect ELISA.

*2.2.1. Virus neutralization testing*

The standard virus neutralization microtiter assay was used to detect serum antibodies against BVDV (21). The BVDV 1 cytopathic strain NADL and BVDV 2 cytopathic strain 125c were used (Walz et al. 2008). Briefly, after heat inactivation at 56°C for 30 minutes, serial 2-fold dilutions (1:2 to 1:4096) were made in 50 μL of culture medium. For each dilution, 3 wells of a 96-well plate (Immulon 4HBX, Thermo Electron Corp, Milford, MS) were inoculated with an equal volume (50 μL) of culture medium containing 100-500 $TCID_{50}$ of the test strain. After inoculation, the plate was incubated at 38.5°C in a humidified atmosphere of 5% $CO_2$ and air for 1 hour. Then, $2.5 \times 10^3$ Madin Darby bovine kidney (MDBK) cells in 50 μL of culture medium were added to each well. Plates were incubated for 72 hours and evaluated visually for cytopathic effect (Walz et al. 2008; Passler et al. 2008). Mean $Log_2$ antibody titers were calculated from the endpoint titers of the animals in each group. Sero-negativity to BVDV 1, and BVDV 2 was defined as a serum antibody titer less than 2 which equates to a $Log_2$ antibody titer of 0. Mean time to reach sero-negative status for BVDV 1 and BVDV 2 and proportion of calves susceptible to acute BVDV infection (serum titer $\leq$ 1:16 or mean $Log_2$ antibody titer $\leq 4$) (Bolin and Ridpath 1992) per time period were calculated and compared between groups.

*2.2.2. Indirect ELISA*

An enzyme-linked immunosorbent assay (ELISA) for antibodies to BoHV-1, BRSV, and PI3V was performed as previously described (Durham et al. 1986; Durham and Hassard 1990). Briefly, 96-well polystyrene microtiter plates (Immulon 4HBX, Thermo Electron Corp, Milford, MS) were coated with BoHV-1, BRSV, and PI3V

antigen diluted in carbonate buffer (pH, 9.6) in alternating rows with cell control (uninfected cell lysate) and incubated overnight at 4°C, then the microtiter plates were washed, and blocked with 0.2% gelatin (Sigma Chemical Co, St Louis, MO) in carbonate buffer. For the test, sera were added at 1:50 dilution (BRSV) or 1:200 (BoHV-1 and PI3) to virus and control wells and incubated at 37°C for 1 hour. Positive and negative control serum was included in each plate. The plates were washed, followed by addition of a 1:5,000 dilution of horseradish peroxidase– conjugated protein G (Zymed, San Francisco, CA). Single component 2,2'-azino- di(3-ethyl-benzthiazoline-6-sulfonate) was used as the enzyme substrate (Kirkegaard & Perry Laboratories Inc, Gaithersburg, MD). Sample and antibody dilutions were made in ELISA working buffer [0.01M phosphate buffer (pH, 7.2) with 0.75M NaCl and 0.05% Tween 20h] with 0.2% gelatin. Standard positive control sera for BoHV-1 and PI3V were obtained from cattle with high neutralizing antibody titers against the respective viruses. Convalescent serum from an unvaccinated calf with naturally occurring BRSV infection was used as a positive control for BRSV. Fetal bovine serum (FBS) was used as negative control for all serum samples. All ELISA results were calibrated against their respective standard positive control sera and a negative control serum (FBS) to give uniformity to the results over the period of time that tests were performed. Optical density (OD) values were measured by use of a microplate reader (Benchmark microplate reader, Bio-Rad Laboratories Inc, Mississauga,ON, Canada) at 492 nm and converted to ELISA units (EU) with a software program (Microplate Manager, version 5.0.1, Bio-Rad Laboratories Inc, Mississauga, ON, Canada). The net OD value of the sample was calculated by subtracting the mean sample OD values of the cell control–coated wells from the mean sample OD values of the

BoHV-1, BRSV, and PI3V antigen-coated wells. Similarly, the net OD values were calculated for the positive and negative control sera (Durham et al. 1986). Final serum results were expressed in EU as follows:

$$\frac{\text{Mean net sample OD} - \text{mean net neg. control OD}}{\text{Mean net pos. control OD} - \text{mean net neg. control OD}} \times 100$$

The results for each virus were classified as positive or negative based on ELISA reactivity greater than 10 EU. This is equivalent to a serum neutralizing-antibody titer of 1:3 for BoHV-1, BRSV, and PI3V (Menateau-Horta et al. 1985). Mean time to reach sero-negative status for BoHV-1, BRSV, and PI3V and proportion of sero-negative calves per time period was estimated and compared between groups.

*2.2.3. Single radial immunodiffusion*

Serum (diluted 1:4), maternal colostrum (diluted 1:15), and colostrum replacement samples (diluted 1:15) were assayed for total IgG concentration by single radial immunodiffusion, essentially as previously described (Chelack et al. 1993). Antiserum against bovine IgG (H and L chain) was used (Jackson Laboratories Inc., West Grove, PA). Single radial immunodiffusion plates were prepared from 2% agarose containing 2.5% antiserum in phosphate buffered saline (PBS pH=7.25). Standard curves (1.06-8.5 g/L) were produced using duplicate samples of a bovine IgG serum calibrator (Midlands Bio Products Corp., Boone, Iowa). The validity of plates was assessed with a reference serum from the Centre for Veterinary Biologics, Animal Poultry Health Inspection Service, United States Department of Agriculture. All samples were tested in triplicate and incubated in a humid atmosphere at 25ºC for 18-24 hours. Ring diameters

were measured with a computer assisted plate reader (The Binding Site, Birmingham, England) and the values in samples calculated using a program for linear analysis.

*2.3 Statistical analysis*

All statistics were calculated using a commercial statistics software program (SAS Institute Inc. Atlanta, GA) with an alpha level of 0.05. To detect the decay in serum antibody titers over time, the mean serum antibody titer for each virus after MC or CR intake measured at 2 days of life was compared with the mean serum antibody titer at 7 days of life, and 1, 2, 3, 4, 5, 6, 7, and 8 months of life by the use of repeated-measures analysis in a mixed generalized linear model. An ANOVA was performed to compare the mean time to reach sero-negative status among treatment groups by the use of a generalized linear model. Since antibody data did not have an approximately normal distribution and variances were not homogeneous between groups, a non-parametric one way ANOVA (Kruskal-Wallis) test was performed to compare means of serum antibody levels for each virus at each time point and to compare serum and maternal colostrum/colostrum replacer IgG concentrations among treatment groups. Additionally, ranges of antibody values and the coefficient of variation (Cv) were calculated at 2 days, 2 months, and 5 months to evaluate the range of antibody values for each virus within group and compare between MC and CR groups.

## Results

*3.1. IgG and apparent efficiency of absorption (AEA)*

Pre-colostral IgG serum levels from all calves were below detectable concentrations. The mean total mass of IgG administered to the calves was higher in maternal colostrum compared with colostrum replacer, 277.4 g vs. 208.5 g ($p = 0.0089$), respectively. Calves in the MC group had higher mean concentrations of serum IgG at 2 days of age compared with calves in the CR group, 20.65 g/L vs. 12.41 g/L ($p = 0.021$), respectively. Additionally, IgG serum concentrations at 2 days of age between calves fed with a bottle and calves fed with an esophageal feeder were not observed within CR or MC groups ($P = 0.098$). The **AEA** at 2 days of age was higher in the MC group compared with the CR group, 27.21% vs. 23.40%, respectively; however, **AEA** was not significantly different between groups ($p = 0.2238$).

*3.2 Level of antibodies to viruses at each time period*

The mean BVDV 1 antibody titer was greater at 2 days, 7 days, and 3 months of age in CR calves compared with calves in the MC group (Figure 1). Similarly, average BVDV 2 antibody titers were significantly higher at 2 days, 7 days, 1, 2, 3, and 4 months of age in CR calves compared with MC calves (Figure 2). The mean BoHV-1 antibody level was greater at 8 months of age in the MC group (mean = 0.47 EU) compared with the CR group (mean = 0.0 EU) ($p = 0.0328$); however, additional differences were not observed (Figure 3). For BRSV, the mean antibody level was significantly higher at 7 days of age in the CR group (mean = 81.3 EU) compared with the MC group (mean = 70.65 EU) ($p = 0.0384$); however, statistically significant differences were not observed at other time points (Figure 4). For PI3V, statistically significant differences were not observed between groups at any time point.

*3.3 Ranges of antibodies to viruses*

The ranges of antibodies to BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V at 2 days, 2 months, and 5 months of age in CR and MC groups are shown in Table 1. In general, the ranges of serum antibodies for BVDV 1 and BVDV 2 at 2 days, 2 months, and 5 months of age were larger in the MC group compared with the CR group. Similarly, the ranges of antibodies against BRSV, BoHV-1, and PI3V were larger, and the Cv higher in the MC group at every time point compared with the CR group.

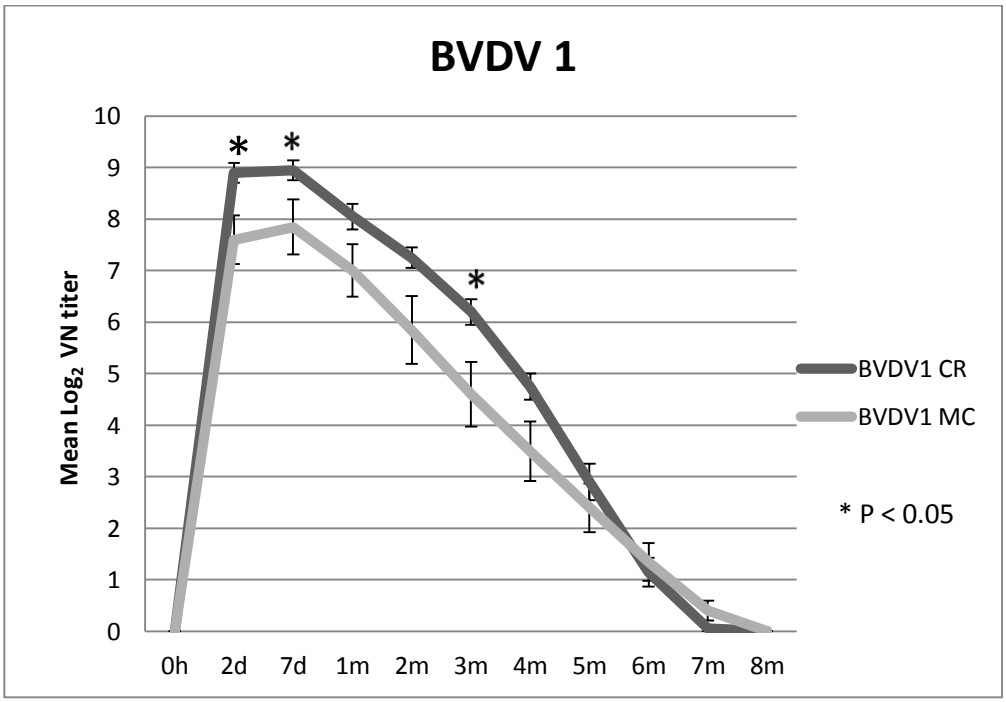


Figure 1. Antibody titers (mean ± SEM) and decay to BVDV 1 between colostrum replacement and maternal colostrum groups during the entire study period. Calves that received colostrum replacement (dark grey) at birth demonstrated higher $Log_2$ VN titer to BVDV 1 at 2 days ($p = 0.0194$), at 7 days ($p = 0.05$), and at 3 months of age ($p = 0.0427$) compared with calves that received maternal colostrum (light grey). The decay rate for antibody titers was similar between groups.

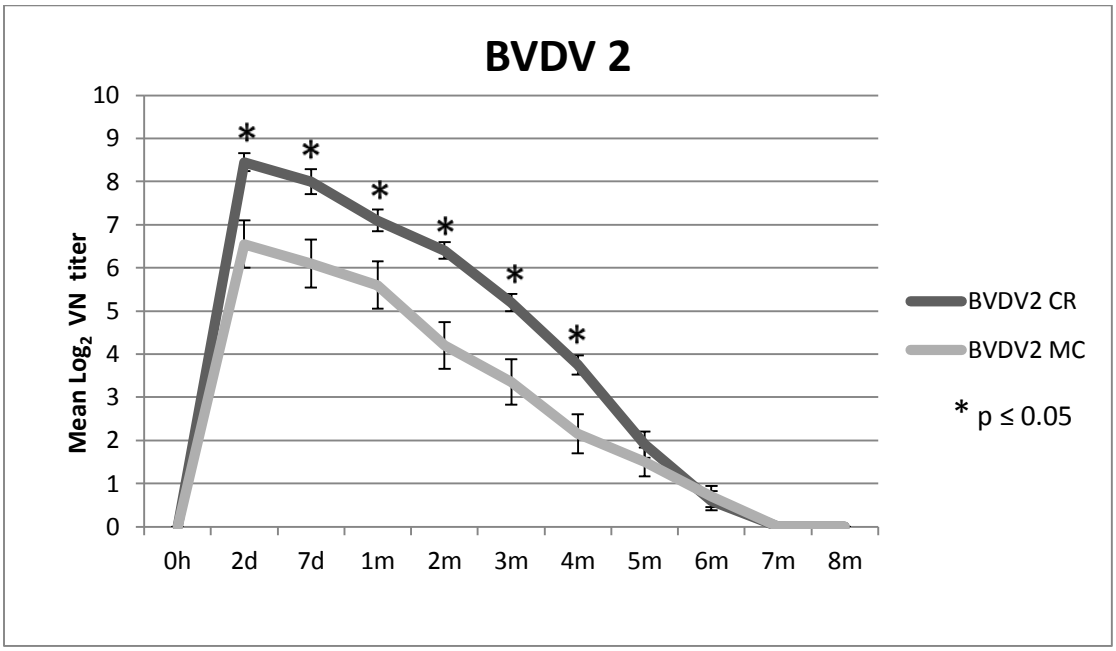


Figure 2. Antibody titers (mean ± SEM) and decay to BVDV 2 between colostrum replacement and maternal colostrum groups during the entire study period. Calves that received colostrum replacement (dark grey) at birth demonstrated higher $Log_2$ VN titer to BVDV 2 at 2 days ($p = 0.0119$), 7 days ($p = 0.0074$), 1 month ($p = 0.05$), 2 months ($p = 0.0024$), 3 months ($p = 0.0098$), and 4 months of age ($P = 0.0107$) compared with calves that received maternal colostrum (light grey). The decay rate for antibody titers was similar between groups.

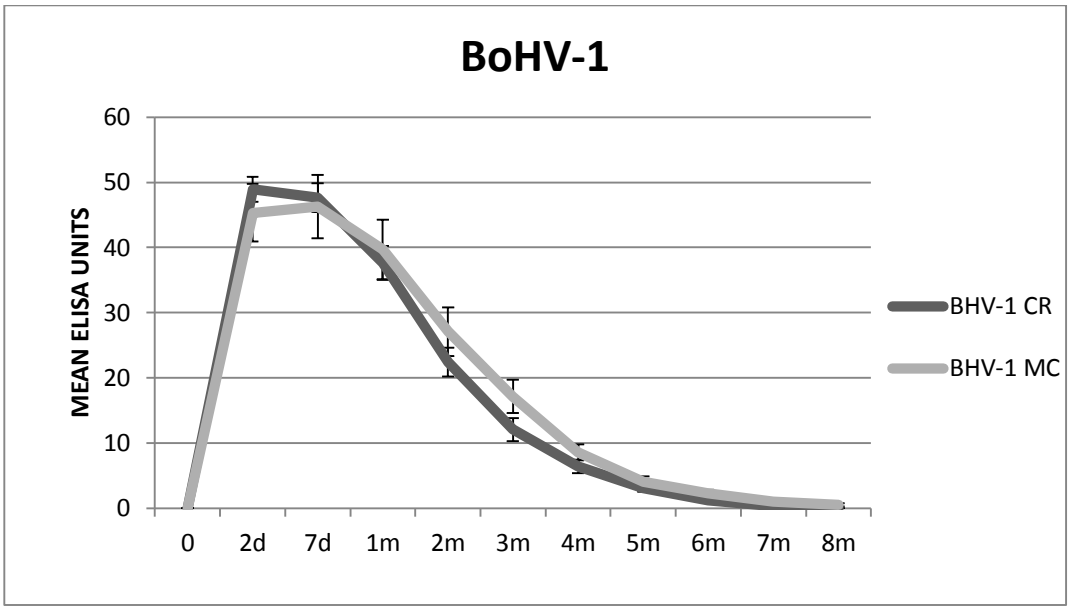


Figure 3. Antibody titers (mean EU ± SEM) and decay to BoHV-1 between colostrum replacement and maternal colostrum groups during the entire study period. Calves that received maternal colostrum (light grey) at birth demonstrated higher mean EU titers to BHV- at 8 months of age compared with calves that received colostrum replacement (dark grey) ($p = 0.032$). The decay rate for antibody titers was similar between groups.

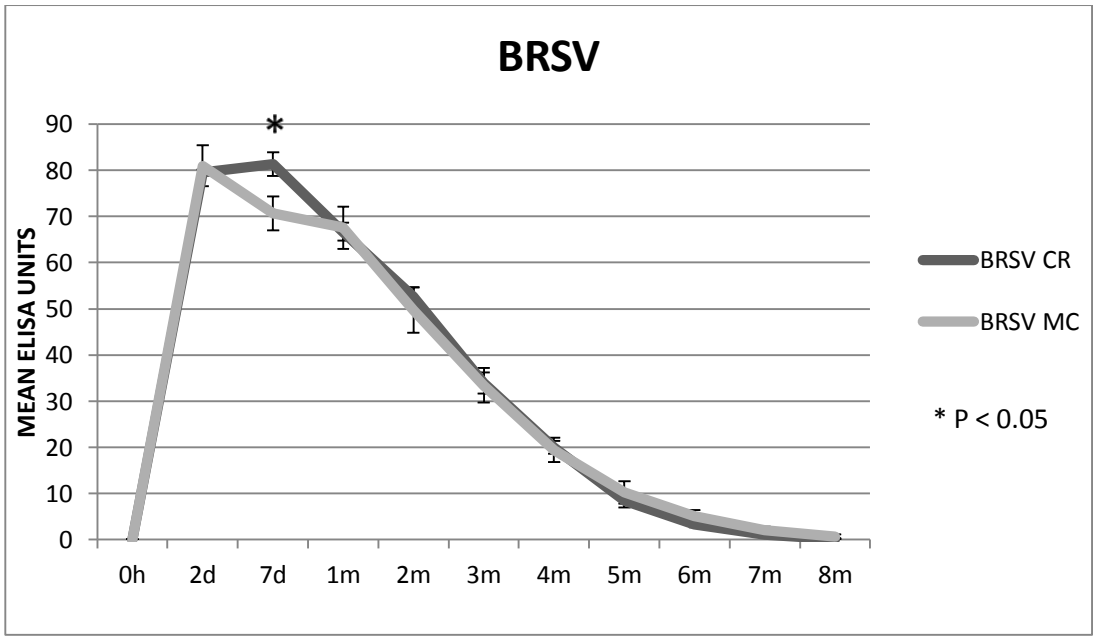

Figure 4. Antibody titers (mean EU ± SEM) and decay to BRSV between colostrum replacement and maternal colostrum groups during the entire study period. Calves that received colostrum replacement (dark grey) at birth demonstrated higher mean EU titers to BRSV at 7 days of age ($p = 0.0384$) compared with calves that received maternal colostrum (light grey). The decay rate for antibody titers was similar between groups.

*3.4 Estimated time to seronegative status*

Duration of passively-derived immunity expressed as the mean time to reach sero-negative status for BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V was not significantly different between CR and MC groups (Table 2). Significant decay of antibody titers was observed after 1 month of age for each virus in both groups and decay rate was similar between groups. However, calves in the MC group became susceptible (Figures 5 and 6) and seronegative to BVDV 1 and BVDV 2 earlier and in greater proportions at each time period compared with CR calves during the first 5 months of age Similarly, calves in the MC group started to become sero-negative to BRSV earlier and in greater proportions at each time period compared with CR calves (Figure 8). Results for BoHV-1 and PI3V were similar between groups, and calves in the MC started to become sero-negative for these viruses earlier in life compared with CR calves (Figure 7).

| Virus | Time point | Colostrum Replacement | | | Maternal Colostrum | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | Cv %[a] | Mean | Range | Cv % |
| | | | | | | | |
| **BVDV 1** | **2 days[b]** | 8.9 | 8-10 | 9.55 | 7.6[c] | 5-11 | 28.03 |
| | **2 months** | 7.25 | 6-9 | 12.55 | 5.85 | 1-11 | 51.11 |
| | **5 months** | 2.9 | 0.5 | 55.52 | 2.4 | 0-5 | 87.92 |
| | | | | | | | |
| **BVDV 2** | **2 days** | 8.45 | 7-10 | 11.12 | 6.55[c] | 2-11 | 37.40 |
| | **2 months** | 6.4 | 5-8 | 13.75 | 4.2[c] | 0-7 | 57.14 |
| | **5 months** | 1.9 | 0-4 | 74.21 | 1.5 | 0-4 | >100 |
| | | | | | | | |
| **BRSV** | **2 days** | 79.5 | 74-84 | 3.50 | 80.95 | 26-119 | 24.98 |
| | **2 months** | 52.65 | 45-70 | 17.59 | 49.8 | 3-97 | 45.32 |
| | **5 months** | 8.45 | 0-21 | 78.46 | 10.3 | 0-44 | >100 |
| | | | | | | | |
| **BoHV-1** | **2 days** | 48.9 | 33-71 | 17.59 | 45.3 | 11-85 | 43.49 |
| | **2 months** | 22.4 | 8-41 | 44.42 | 27.1 | 0-52 | 61.48 |
| | **5 months** | 3.05 | 0-3 | >100 | 4.0 | 0-9 | >100 |
| | | | | | | | |
| **PI3V** | **2 days** | 124.8 | 83-187 | 20.06 | 106.6 | 22-168 | 34.19 |
| | **2 months** | 68.15 | 28-134 | 35.72 | 63.15 | 4-128 | 55.88 |
| | **5 months** | 5.1 | 0-23 | >100 | 8.0 | 0-37 | >100 |

Table 1. Antibody titer means ($Log_2$ antibody titer for BVDV 1 and BVDV 2 and EU for BRSV, BoHV-1, PI3V) to respiratory viruses with their respective ranges and coefficient of variation at 3 different time points in CR and MC groups.

[a] Coefficient of variation ($Cv=\sigma/\mu *100$) of the mean of antibody titers for each virus at each time point. [b] Time point 2 days represent the first sample after maternal colostrum or colostrum replacement administration. [c] Statistical significance between colostrum replacement and maternal colostrum mean titers $P < 0.05$

| Virus | Mean time to sero-negative status ± SD[a] in months | |
|---|---|---|
| | **Colostrum Replacement** | **Maternal Colostrum** |
| **BVDV 1** | 6.1 ± 0.7 | 5.5 ± 1.7 |
| **BVDV 2** | 6.5 ± 0.6 | 6.1 ± 1.6 |
| **BRSV** | 5.5 ± 0.6 | 5.2 ± 1.5 |
| **BHV-1** | 3.75 ± 0.8 | 3.8 ± 1.6 |
| **PI3V** | 5.1 ± 0.8 | 4.9 ± 1.66 |

Table 2. Mean time to reach sero-negative status to BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V. [a] Standard deviation of the mean time to sero-negative status in months for each virus in maternal colostrum and colostrum replacement groups.

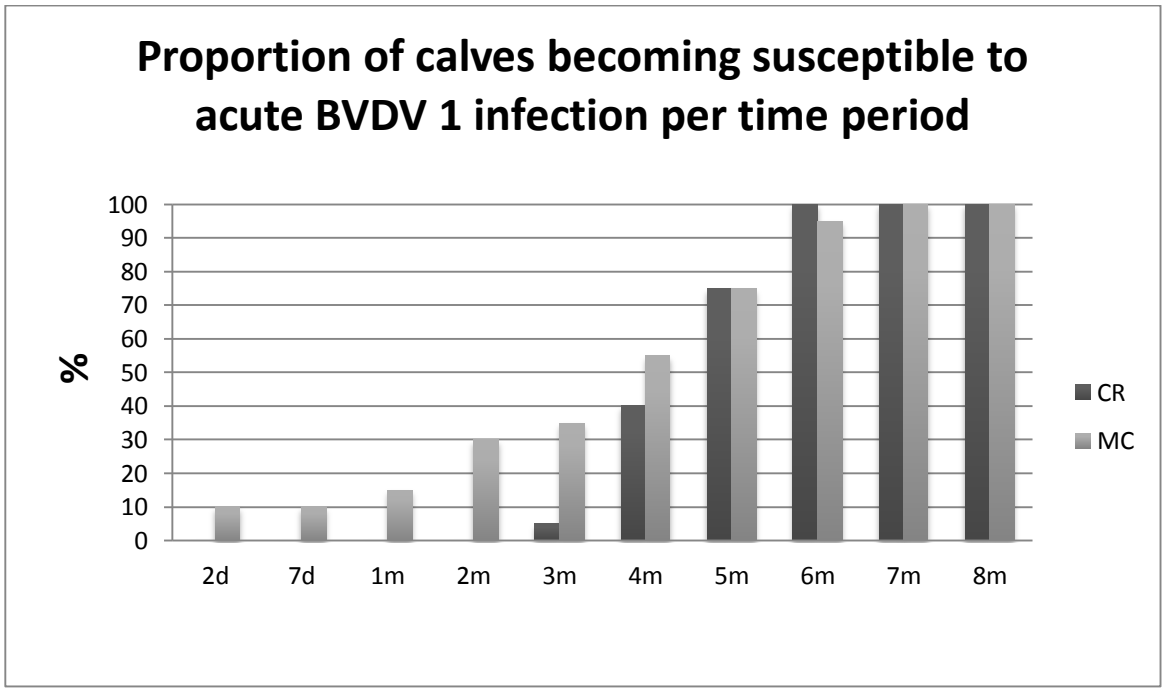


Figure 5. Proportion of calves becoming susceptible to acute BVDV 1 infection ($Log_2$ antibody titer $\leq 4$) per time period between colostrum replacement and maternal colostrum groups. During the first 5 months calves in the maternal colostrum group (light grey bars) started to become susceptible to BVDV 1 infection earlier and in greater

proportions per time period compared with calves from the colostrum replacement group (dark grey bars).

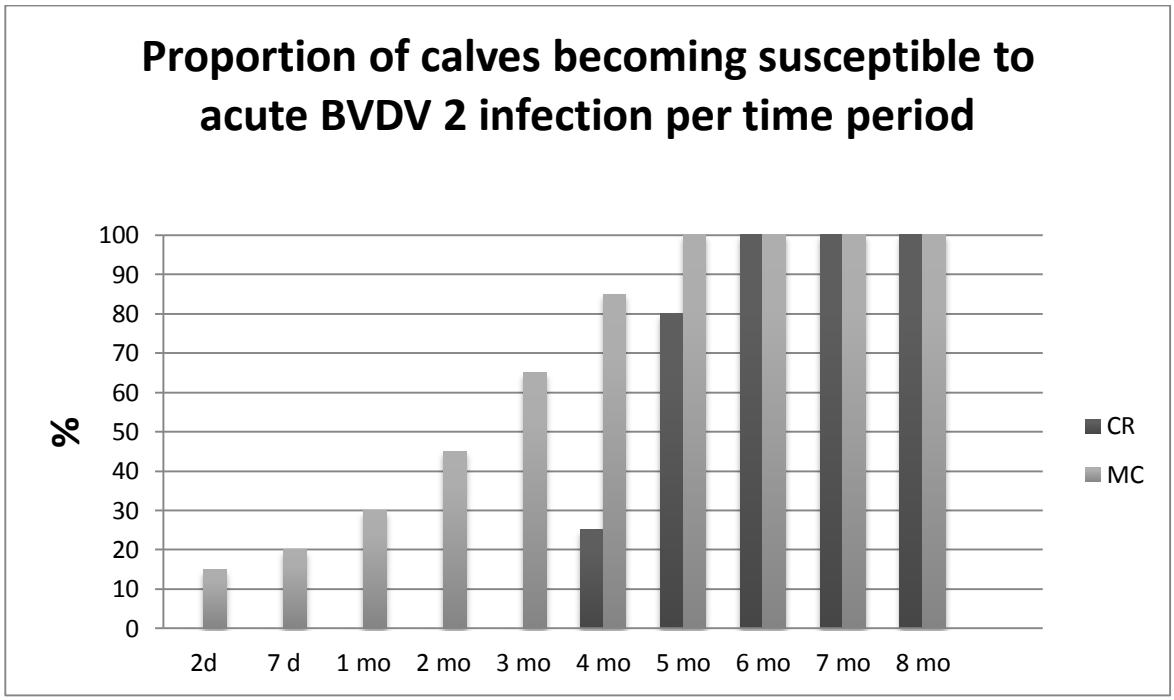


Figure 6. Proportion of calves becoming susceptible to acute BVDV 2 infection ($Log_2$ antibody titer $\leq 4$) per time period between colostrum replacement and maternal colostrum groups. Calves in the maternal colostrum group (light grey bars) started to become susceptible to BVDV 2 infection earlier and in greater proportions per time period compared with calves from the colostrum replacement group (dark grey bars).

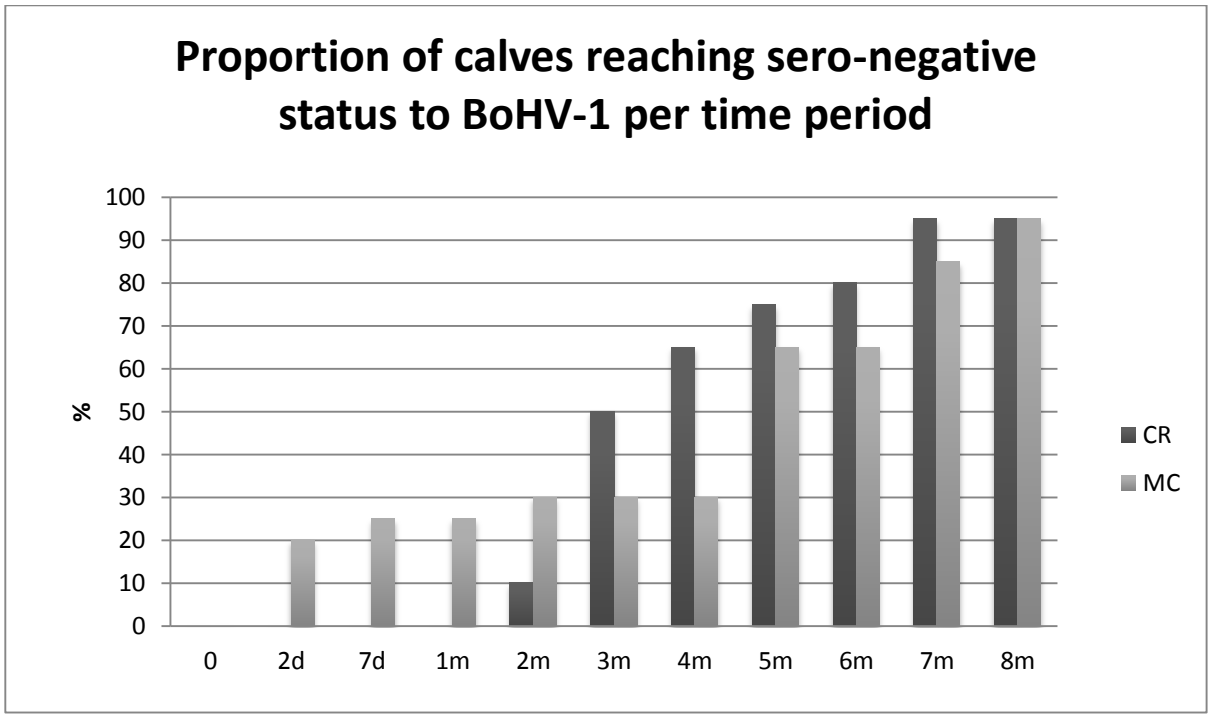


Figure 7. Proportion of calves reaching sero-negative status to BHV-1 (serum EU ≤ 10) per time period between colostrum replacement and maternal colostrum groups. Calves in the maternal colostrum group (light grey bars) started to become sero-negative to BHV-1 earlier in life compared with calves from the colostrum replacer group (dark grey bars).

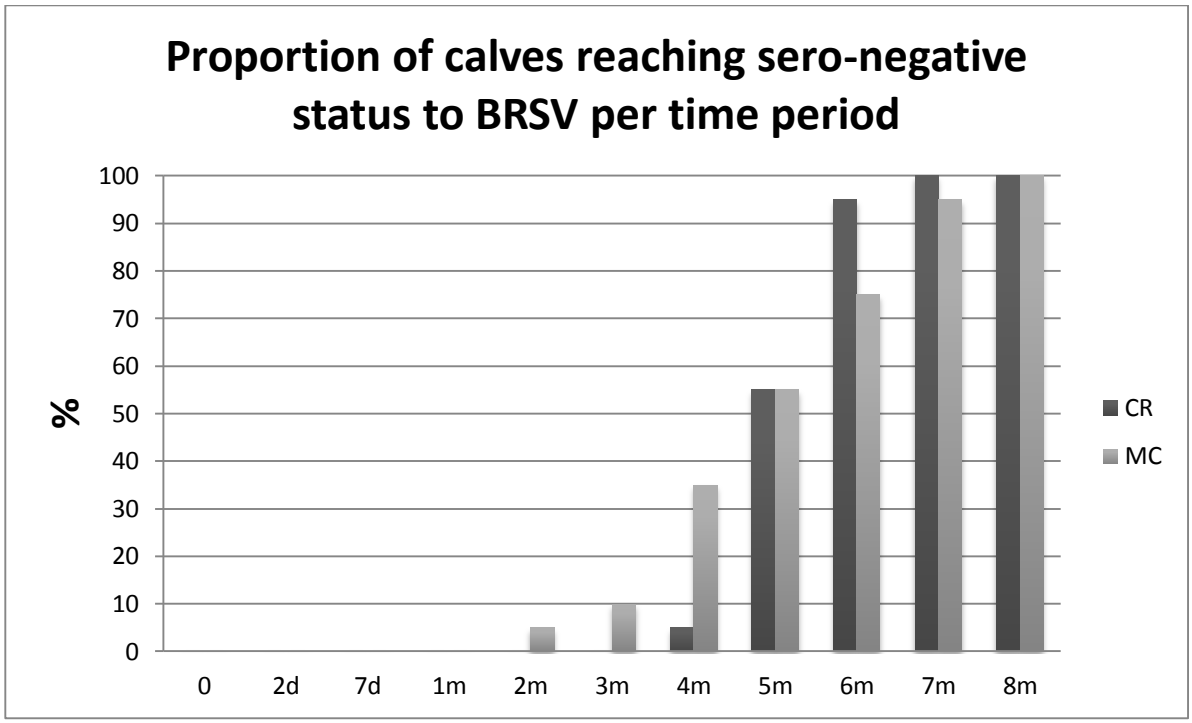


Figure 8. Proportion of calves reaching sero-negative status to BRSV (serum EU ≤ 10) per time period between colostrum replacement and maternal colostrum groups. During the first 5 months calves in the maternal colostrum group (light grey bars) started to become sero-negative to BRSV earlier and in greater proportions per time period compared with calves from the colostrum replacement group (dark grey bars).

## Discussion

This is the first report demonstrating the efficacy of a colostrum replacement product to provide neonatal calves with adequate serum levels and duration of passive immunity against common respiratory viruses including BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V. The results indicate that this colostrum replacement product provides neonatal calves with serum levels of virus-specific antibodies to BVDV 1, BVDV 2,

BRSV, BoHV-1, and PI3V similar to those in calves that receive 3.8 L of good quality maternal colostrum (Munoz-Zanzi et al. 2002; Fulton et al. 2004). In addition, calves that received the CR had greater mean serum antibody titers to BVDV 1 and BVDV 2 during the first 4 months of age. High mean concentration of BVDV-specific antibodies present in the colostrum replacement product and low BVDV-specific antibody concentrations in maternal colostrum could have contributed to the greater serum antibody titers to BVDV observed in the CR group. The concentrations of BVDV-specific antibodies in maternal colostrum could have been influenced by multiple factors such as the degree of exposure of cattle to field strains of BVDV within the herd, vaccination program of the herd, frequency of vaccination, adequate response to vaccination, and ability to transfer specific antibodies to colostrum (Menateau-Horta et al. 1985; Kirkpatrick et al. 2001; Munoz-Zanzi et al. 2002). The levels of antibodies in the CR product, in contrast, would be expected to be much more uniform given that the product is produced from pools of large numbers of individual colostra which are selected based on IgG concentration.

Comparison of the variation in virus specific antibodies at different time periods demonstrated a greater variability among levels of BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V antibodies in calves in the MC group compared to calves in the CR group. In general, higher variability of antibody levels and greater coefficients of variation were observed at 2 days, 2 months, and 5 months of age in the MC group. A wide range of antibody titers to respiratory viruses has been previously reported in calves after maternal colostrum intake (Kirkpatrick et al. 2008; Fulton et al. 2004). The large variation in levels of colostrum-derived antibodies to respiratory viruses after maternal colostrum intake has a direct effect on the duration of colostrum-derived immunity, as calves with low initial

virus-specific antibodies absorbed from colostrum become sero-negative and susceptible to acute viral infection earlier in life (Munoz-Zanzi et al. 2002; Kirkpatrick et al. 2001). Factors such as diversity of maternal colostrum sources, differences in concentration of virus-specific antibodies in maternal colostrum, and prevalence of FPT influence the levels of colostrum-derived antibodies in calves fed maternal colostrum; however, the mean serum IgG concentrations at 2 days of life in both the MC and CR groups were above the IgG concentration in serum associated with FPT in calves (< 10 g/L) (Weaver et al. 2000) and therefore FPT was unlikely as a factor contributing to the variability in antibody levels observed in the MC group. The lower AEA observed in the CR group compared with the MC group, and compared with results of the use of this product in previous studies (Godden et al. 2009a; Godden et al. 2009b) could have been the result of using more water when reconstituting the colostrum replacement product than what manufacturer recommended.

Several studies have reported the duration of detection of maternal colostrum-derived antibodies against BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V as the mean time to reach sero-negative status for each virus (Munoz-Zanzi et al. 2002; Fulton et al. 2004; Kirkpatric et al. 2008). The longevity of passively-derived immunity in calves that receive maternal colostrum at birth is highly variable (Menateau-Horta et al. 1985; Van der Poel et al. 1999; Kirkpatrick et al. 2001; Fulton et al. 2004). In one study, the estimated mean time to reach sero-negative status for respiratory viruses in a group of calves that had received maternal colostrum was 117.7 ± 37.7 days for BVDV 1, 93.9 ± 61.9 days for BVDV 2, 200.2 ± 116.7 days for BRSV, 65.1 ± 37.8 days for BoHV-1, and 183.8 ± 100.0 days for PI3V (Fulton et al. 2004). Another study reported that the mean

duration for colostrum-derived antibodies in beef calves was 185.6 ± 59.8 days for BVDV 1, 157.8 ± 56.1 days for BVDV 2, 122.9 ± 46.6 days for BoHV-1, 190.6 ± 58.3 days for PI3V, and 183.7 ± 33 days for BRSV (Kirkpatrick et al. 2001). In the present study, the mean time to reach sero-negative status to BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V in CR and MC calves varied with virus but was similar for each virus between groups; however, the variability in the duration was larger in the MC group compared with the CR group. While the standard deviation for time to sero-negative for BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V in the MC group ranged from 1.5 months (45 days) to 1.7 months (53 days) the standard deviation for the same viruses in the CR group ranged from 0.6 months (18 days) to 0.8 months (24 days). Additionally, when the proportion of calves becoming sero-negative and susceptible to virus infection per time period was evaluated, calves from the MC group started to become susceptible to BVDV infection and sero-negative to all viruses at earlier time periods and in most cases, at greater proportions compared with CR calves. Variable duration of colostrum-derived immunity and differences in the proportion of animals becoming susceptible or sero-negative to viral infection could result in poor calf-herd immunity and increase the risk of introduction of infectious pathogens such as BVDV, BRSV, BoHV-1, and PI3V into the calf-herd. Variability in the duration of colostrum-derived immunity against various respiratory viruses has been related to multiple factors including differences in the rate of decay of colostrum-derived antibodies (Van der Poel et al. 1999; Kirkpatrick et al. 2001; Munoz-Zanzi et al. 2002; Fulton et al. 2004), which is usually influenced by active viral infections or vaccination. The decay rate of passively-derived neutralizing antibodies in this study was similar between groups. Calves were not vaccinated at any

time point, and sero-conversion was not observed to any of the viruses, so the possibility of an active viral infection was unlikely. In the present study, the variation in the levels of colostrum-derived antibodies against respiratory viruses at 2 days of life observed in MC calves was likely responsible for the variation in the times to reach sero-negative status within the MC group.

The results of this study indicate that calves that receive timely and adequate amounts of high quality MC at birth absorb amounts of IgG into the serum well above the threshold defined for FPT but still demonstrate highly variable levels of maternal immunity to individual respiratory viruses including BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V. This results in variable times at which calves become sero-negative or susceptible to acute viral infection and disease. In contrast, the colostrum replacement product used in this study provides calves with adequate passive transfer and less variable levels of passive immunity to BVDV 1, BVDV 2, BRSV, BoHV-1, and PI3V which results in more uniformity of the time at which calves become susceptible and sero-negative to viral pathogens. The present study did not attempt to evaluate the ability of passive immunity derived from maternal colostrum or colostrum replacement to protect calves against challenge with virulent strains of respiratory viruses, nor the antibody levels associated with protection against each virus. Nevertheless, based on our findings, it is reasonable to suggest that overall, calves fed this CR product will have better duration of immunity compared with calves fed the more variable MC and therefore vaccination programs against respiratory viruses should start earlier in calves that receive maternal colostrum as they could become susceptible to acute viral infection and disease earlier in life. The more uniform time to sero-negativity suggests that it should be

possible to better predict the optimum time to vaccinate calves fed the CR used in this study compared with MC and this should be the subject of additional study.

Efficacy of multivalent, modified live virus (MLV) vaccines administered to early weaned beef calves subsequently challenged with virulent *Bovine viral diarrhea virus* type 2


## Abstract

Vaccination of young calves against *Bovine viral diarrhea virus* (BVDV) is desirable in dairy and beef operations to reduce clinical disease and prevent spread of the virus among cattle. Although protection from clinical disease by multivalent, modified-live virus (MLV) vaccines has been demonstrated, the ability of MLV vaccines to prevent viremia and viral shedding in young calves possessing passive immunity is not known. The purpose of this study was to compare the ability of three different MLV vaccines to prevent clinical disease, viremia, and virus shedding in early weaned beef calves possessing maternal immunity that were vaccinated once 45 days prior to challenge with virulent BVDV 2 . At 45 days following vaccination, calves that received vaccines B and C had significantly higher BVDV 1 and BVDV 2 serum antibody titers compared with control calves. Serum antibody titers for BVDV 1 and BVDV 2 were not significantly different between control calves and calves that received vaccine D. Following BVDV 2 challenge, a higher proportion of control calves and calves that received vaccine D presented viremia and shed virus compared with calves that received vaccines B and C. Rectal temperatures and clinical scores were not significantly different between groups at

any time period. Calves that received vaccines B and C had significantly higher mean body weights at BVDV 2 challenge and at the end of the study compared with control calves. Moderate to low maternally-derived BVDV antibody levels protected all calves against severe clinical disease after challenge with virulent BVDV 2. Vaccines B and C induced a greater antibody response to BVDV 1 and BVDV 2, and resulted in reduced viremia and virus shedding in vaccinated calves after challenge indicating a greater efficacy in preventing virus transmission and reducing negative effects of viremia.

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## Introduction

*Bovine viral diarrhea virus* (BVDV) is an important cause of respiratory, enteric, and reproductive disease in cattle and has been associated with major economic losses in cattle operations worldwide (Houe 1995). Vaccination of young calves against BVDV reduces the number of acute infections in the herd and limits spread of virus among cattle populations (Houe 1995; Ridpath 2013); however, effective vaccination of young calves against BVDV can be challenging due to the presence of maternally-derived BVDV antibodies at the time of vaccination (Cortese et al. 1998c). Although maternally-derived BVDV antibodies can provide protection against acute BVDV infection and clinical

disease, humoral immune responses to vaccination might be adversely affected (Ellis et al. 2001). Concentration of maternally-derived BVDV antibodies and age of calf at the time of vaccination are important factors in the induction of adequate immune responses following BVDV immunization (Menanteau-Horta et al. 1985; Munoz-Zanzi et al. 2002; Fulton et al. 2004). Calves with moderate to high maternally-derived BVDV antibody levels at vaccination do not usually respond with an increase in BVDV antibodies but are protected against clinical disease, have a slower decay rate of maternal immunity, and develop anamnestic antibody responses following BVDV challenge (Howard et al. 1989; Platt et al. 2009; Woolums et al. 2013). Calves with low maternally-derived BVDV antibody levels respond to vaccination by increasing BVDV antibody titers. The priming of naive B and T cells, the induction of specific cell mediated immune memory responses, and the induction of anamnestic antibody responses have been identified as the main source of protection of young calves vaccinated in the presence of maternally-derived antibodies and subsequently challenged with virulent BVDV (Endsley et al. 2002; Ridpath et al. 2003; Endsley et al. 2003; Endsley et al. 2004; Zimmerman et al. 2006).

Early weaned beef calves possess variable levels of maternally-derived BVDV antibodies at 2–4 months of age and therefore could benefit from vaccination prior to stress of weaning and shipment. A single dose of a multivalent, MLV BVDV vaccine was demonstrated to be effective in protecting young calves possessing different levels of maternal immunity against acute BVDV infection (Step et al. 2009). In addition to prevention of clinical disease, vaccination should limit the spread of BVDV by reducing virus shedding and horizontal transmission, a desirable outcome of vaccination in herds

or units where populations of highly stressed cattle are commingled. However, experimental studies comparing different multivalent MLV vaccines containing BVDV in their ability to prevent viremia and viral shedding in young calves possessing maternally-derived immunity that subsequently undergo challenge with virulent BVDV are limited. The objective of this study was to evaluate the ability of three different commercially available, multivalent MLV vaccines containing BVDV to prevent clinical disease and reduce shedding of virus when administered to early weaned beef calves subsequently challenged with virulent BVDV 2 at 45 days after vaccination.

## Materials and Methods

### *2.1. Animals*

Forty-eight crossbred steer calves born and raised at the Upper Coastal Plain Agricultural Research Center, Winfield, AL were utilized in this study. Calves were born in September-October 2012 to cows that had received at least one dose of a modified-live BVDV vaccine D[a] prior to breeding during the 2 years previous to the start of the study. At birth, calves were identified by an ear tattoo and ear tag. All calves remained with their dams and consumption of colostrum occurred under natural conditions in the pasture. A blood sample for detection of neutralizing antibodies against BVDV 1 and BVDV 2 was collected from all calves at day −75 of the study at a median calf age of 44 days to determine the initial maternally-derived BVDV antibody levels.

### *2.2. Experimental design*

All calves were early weaned on study day −45, which corresponded to calf ages between 62–92 days (2–3 months) with a median age of 72.2 days. To prepare for early weaning, creep feeding was offered for 3 weeks prior to weaning in order to train calves to the weaning diet. The weaning diet was an energy dense (65-75% of total digestible nutrients), relatively high protein (14-17%), and highly palatable ration to meet all nutritional requirements of young growing calves. Calves were stratified by initial maternally-derived BVDV 2 serum antibody titers and assigned by the use of a random number generator [b] to 1 of 4 different vaccination groups. The stratification by initial levels of BVDV 2 antibodies ensured that each group received similar numbers of calves with different levels of maternally-derived BVDV 2 antibody levels. All calves underwent abrupt weaning on day −45 of the study and were vaccinated according to their assigned treatment group A (n = 12), B (n = 12), C (n = 12), or D (n = 12). Following vaccination, calves were separated in isolated pastures to prevent transmission of vaccine strains between groups. During this time, daily observation of the calves was performed to evaluate for adverse vaccine reactions. Forty-four days after weaning, calves were transported 192 miles to the North Auburn BVDV Unit located in Auburn, AL. Upon arrival to the North Auburn BVDV Unit, calves were rested and given access to fresh water, hay, and supplement. On the next day (day 0), all calves were challenged with virulent BVDV 2 1373 and placed in the same pasture for the remainder of the study. Clinical evaluation and sampling of calves was performed until day 28 after challenge. All calf protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University (PRN # 2012–2157).

*2.3. Vaccines*

All vaccines used were commercially available, USDA-licensed stock material and were administered to calves at weaning (day −45). Group A was the control group and received 2 mL of 0.9% phosphate buffered saline subcutaneously once. Group B received 2 mL of vaccine B[c] subcutaneously once, group C received 2 mL of vaccine C[d] subcutaneously once, and group D received 2 mL of vaccine D[a] subcutaneously once. All vaccines were modified-live and multivalent containing modified-live types 1 and 2 of BVDV, parainfluenza virus 3, bovine herpesvirus 1, and bovine respiratory syncytial virus. All calves in this study were under 6 months of age and vaccination with vaccines B, C, and D was considered off-label.

*2.4. BVDV challenge*

Forty-five days after vaccination (day 0), all calves were experimentally inoculated with the noncytopathic (NCP) BVDV 2 strain 1373. The NCP BVDV 2 strain 1373 has been previously used in experimental BVDV inoculation of calves and shown to induce severe clinical disease, leukopenia, and thrombocytopenia (Brock et al. 2007). The BVDV 2 strain 1373 was propagated in Madin-Darby bovine kidney (MDBK) cells in minimum essential medium [j] (MEM), supplemented with 10% equine serum, L-glutamine, penicillin G (100 units/ml), and streptomycin (100 μg/ml). Virus was harvested from cells by a single freeze-thaw method, aliquoted, and stored (−80°C) until needed. Aliquots were enumerated using the method of Reed and Muench (Reed and Muench 1938), prior to inoculation of calves. All calves were inoculated by intranasal aerosol administration of $1 \times 10^6$ $TCID_{50}$ of the BVDV 2 strain 1373.

*2.5. Sample and data collection*

Daily clinical observations were performed by the same person, who was blinded to study group allocation, on days 0, 3, 6, 8, 10, 14, 21, and 28. Additionally, individual rectal temperatures, serum, whole blood, and deep nasal swab samples were collected on those same days. Whole blood samples were subjected to hematologic analysis on day 0 prior to challenge and on days 6, 8, 10, and 28 after challenge for individual white blood cell and platelet counts.

During sampling days, each calf was scored prior to handling for signs of abnormal respiration, diarrhea, and depression using a scale of 0 to 3, with the absence of a clinical sign scored as 0 and the most severe clinical sign scored as 3 (Stevens et al. 2011). Briefly, an abnormal respiratory score was given if an animal presented with a cough, labored breathing, nasal, or ocular discharge. Nasal and ocular discharges were judged as being serous, mucous, or mucopurulent. Diarrhea scores were judged as being normal feces, pasty feces, runny feces, or severe diarrhea with or without blood. Depression scores ranged from no depression, mild depression, moderate depression, or severe depression. In addition to visual examination, individual body weights were obtained on day −45 (vaccination/weaning day), day 0 (challenge day), and days 14, and 28 after challenge using a portable livestock electric scale [f] that was validated prior to and after each weighing.

*2.6. Virus isolation (VI)*

Whole blood, serum, and deep nasal swab samples collected on days 0, 3, 6, 8, 10, 14, 21, and 28 after challenge were used for BVDV VI using the immunoperoxidase

monolayer assay with techniques previously described (Walz et al. 2008). Briefly, the isolated samples were suspended in 24-well plates and subsequently seeded in 50 µL culture medium. The cell suspension was subjected to co-cultivation on 25 $cm^3$ flasks containing monolayers of MDBK cells and was incubated for 24 hours at 38.5°C and 5% $CO_2$. Following cultivation, 50 µl of the cell culture supernatant was inoculated in triplicate into wells on 96-well microtiter plates containing monolayers of MDBK cells in culture medium. After 96 hours of incubation at 38.5°C and 5% $CO_2$, all samples were frozen at −80°C and subsequently thawed to detect BVDV using the immunoperoxidase monolayer assay as previously described (Walz et al. 2008).

*2.7 Virus neutralization (VN)*

The standard virus neutralization microtiter assay was used to detect antibodies against BVDV in serum of calves collected on days −75, −45, 0 (prior to challenge), and 28 [28]. The BVDV 1 cytopathic strain NADL and BVDV 2 cytopathic strain 125c were used. For samples collected on days 0 and 28, the challenge BVDV 2 1373 non-cytopathic strain was also used. Briefly, following heat inactivation at 56°C for 30 minutes, serial 2-fold dilutions (1:2 to 1:4096) were made in 50 µL of culture medium. For each dilution, 3 wells of a 96-well plate were inoculated with an equal volume (50 µL) of culture medium containing 100–500 $TCID_{50}$ of the test strain. After inoculation, the plate was incubated at 38.5°C in a humidified atmosphere of 5% $CO_2$ and room air for 1 hour. Then, $2.5 \times 10^3$ MDBK cells in 50 µL of culture medium were added to each well. Plates were incubated for 72 hours and evaluated visually for cytopathic effect (Walz et al. 2008; Larska et al. 2013). The geometric mean of antibody titers was

calculated from the endpoint $Log_2$ titers of the animals in each group. Seronegativity to BVDV 1 and BVDV 2 was defined as a serum antibody titer less than 2 which equates to a $Log_2$ antibody titer of 0.

*2.8 Statistical analysis*

All statistical analyses were performed using the GLIMMIX procedure in the SAS 9.3 software package [g]. To detect changes in $Log_2$ transformed antibody levels, virus isolation, rectal temperatures, white blood cell counts, platelet counts, and body weights, a repeated measures generalized linear mixed model [Response = Group + Time (day) + Group * Day] was performed using an appropriate distribution function. The repeated nature of this experiment, viz. multiple observations on the same experimental unit = animal over time, implies non-independence of residuals. Hence, the residual variance was modelled to arrive at a reasonable residual covariance structure using Akaike's Information criterion corrected for small sample sizes (AICc) to determine the best structure. A first-order autoregressive structure with heterogeneous variances (ARH 1) was most commonly fitted. This structure allows for a separate residual variance at each time point and a correlation among time points that diminishes with the lag. Because clinical scores for respiratory distress and diarrhea were binary in nature (only scores 1 and 2 were given) the binary distribution function was used in the abovementioned procedure; this analysis approach is commonly referred to as logistic regression. No analysis was performed for clinical depression score as all scores equalled zero. Dunnett's test for multiple comparisons was used to detect differences between vaccinated groups and the control group; a probability of $P \leq 0.05$ was considered

statistically significant for all tests. The FREQ procedure in the abovementioned software package [h] was used to analyze the proportion of calves with viremia and virus shedding with a $\chi^2$ test.

## Results

### *3.1 Serum virus neutralization titers*

Thirty days prior to vaccination (day −75) and at a median calf age of 44 days, the mean levels of maternally-derived BVDV 1 NADL and BVDV 2 125c serum antibodies were similar between groups (Table 3). At vaccination (day −45), a significant effect of time (day) was detected (P = 0.0001) as decay of maternally-derived antibodies for BVDV 1 NADL and BVDV 2 125c occurred in all groups; however, mean levels of BVDV 1 and BVDV 2 antibodies were not significantly different (P > 0.05) between groups. Forty-five days after vaccination, which corresponded to the time of BVDV 2 challenge (day 0), a significant effect of group and time (day) was detected (P = 0.0001 and P = 0.0001, respectively). Groups B and C calves had mean levels of serum BVDV 1 NADL antibodies significantly greater than controls (P = 0.0033 and P = 0.0002, respectively; Figure 9). Additionally, on day 0, mean levels of BVDV 1 NADL antibodies were similar between group D and the control group (P = 0.2641). With respect to BVDV 2 125c, group C calves had mean levels of antibodies significantly greater than the control group (P = 0.0368; Figure 10). Twenty eight days following challenge, a significant effect of time (day) was detected (p = 0.0001) with respect to the levels of BVDV 2 125c. The mean BVDV 2 125c serum antibody levels increased

similarly in all groups (P > 0.05); in contrast, time (day) and group effects were significant (P = 0.0001 and P = 0.0001, respectively) for BVDV 1 NADL mean antibody levels as these were higher in group B and C calves compared to the control group (P < 0.00001). When antibodies to the challenge strain BVDV 2 1373 were examined at day 0, a significant effect of group and time (day) was detected (P = 0.0001 and P = 0.0001). Calves from groups B and C had greater levels of antibodies compared with control calves (P = 0.05 and P = 0.025, respectively; Figure 11). At day 28 after challenge, a significant (P = 0.0001) effect of time (day) was detected as all groups had increased and similar levels of antibodies to the challenge BVDV strain (P > 0.05).

| | | **Day of Study** | | | |
|---|---|---|---|---|---|
| **Virus** | **Group** | **−75** | **- 45** | **0** | **28** |
| **BVDV 1 NADL** | A | 157.58. (31.12 – 831.74) | 60.12. (14.52 – 250.73) | 15.03. (5.93 – 38.05) | 35.75. (17.38 – 74.02) |
| | B | 135.29$^{NS}$. (28.64 – 639.14) | 67.64$^{NS}$. (17.50 – 259.57) | **107.63**$^{*}$. (62.68 – 183.54) | **861.07**$^{*}$. (377.41 - 1951) |
| | C | 222.86$^{NS}$. (67.64 – 765.36) | 101.12$^{NS}$. (30.27 – 337.79) | **202.25**$^{*}$. (110.66 – 369.64) | **680.28**$^{*}$. (225.97 - 2048) |
| | D | 194.01$^{NS}$. (45.56 – 903.88) | 95.67$^{NS}$. (22.47 – 407.31) | 36.25$^{NS}$. (12.04 – 108.38) | 57.70$^{NS}$. (12.99 – 213.78) |
| **BVDV 2 125c** | A | 107.63. (23.10 - 498) | 47.83. (13.08 – 173.64) | 14.22. (4.08 – 49.18) | 608.87. (292.03 – 1260.69) |
| | B | 101.12$^{NS}$. (23.26 – 439.58) | 60.12$^{NS}$. (15.67 – 232.32) | 42.52$^{NS}$. (22.47 – 80.44) | 809$^{NS}$. (455.08 – 1438.15) |
| | C | 128$^{NS}$. (38.85 – 418.76) | 85.03$^{NS}$. (26.53 – 272.47) | **60.12**$^{*}$. (26.35 – 138.14) | 1820.34$^{NS}$. (955.42 – 3468.26) |
| | D | 128$^{NS}$. (40.78 – 398.93) | 90.50$^{NS}$. (31.34 – 259.57) | 29.85$^{NS}$. (15.24 – 58.89) | 286.02$^{NS}$. (149.08 – 1541.37) |
| **BVDV 2 1373** | A | _ | _ | 16.91. (5.02 – 56.44) | 861.07. (424.61 – 1734.13) |
| | B | _ | _ | **76.10**$^{*}$. (47.50 – 121.09) | 643.59$^{NS}$. (352.13 – 1176.26) |
| | C | _ | _ | **93.05**$^{*}$. (49.18 – 176.06) | 1530.72$^{NS}$. (617.37 – 3795.30) |
| | D | _ | _ | 56.10$^{NS}$. (28.44 – 11.43) | 544.95$^{NS}$. (245.57 – 1200.98) |

Table 3. Geometric mean (95% CI) of virus neutralizing serum antibody titers to BVDV 1, BVDV 2, and BVDV 2 1373 from vaccinated (B, C, and D) and unvaccinated (A) calves at each time period. NS, * = Means within BVDV strain and Day of Study are not significantly different (NS) from the control group A or are significantly different (*) based on Dunnett's test at $P = 0.05$.

Among vaccinated groups, seroconversion, as defined as a 4-fold or greater rise in antibody titers to BVDV 1 NADL after vaccination (at day 0) was observed in 16.6% of the calves from group B, 50% of the calves from group C, and 9% of the calves from group D. Similarly, seroconversion to BVDV 2 125c after vaccination was observed in 16.6% of the calves from group B, 8.3% of the calves from group C, and 9% of the calves from group D. None of the calves in group A (control) seroconverted to BVDV 1 NADL or BVDV 2 125c. The proportion of calves that seroconverted to BVDV 2 125c after vaccination was not significantly different between groups ($P > 0.05$); however, a higher proportion of calves from group C (50%) seroconverted to BVDV 1 NADL with a fourfold increase in antibody titers.

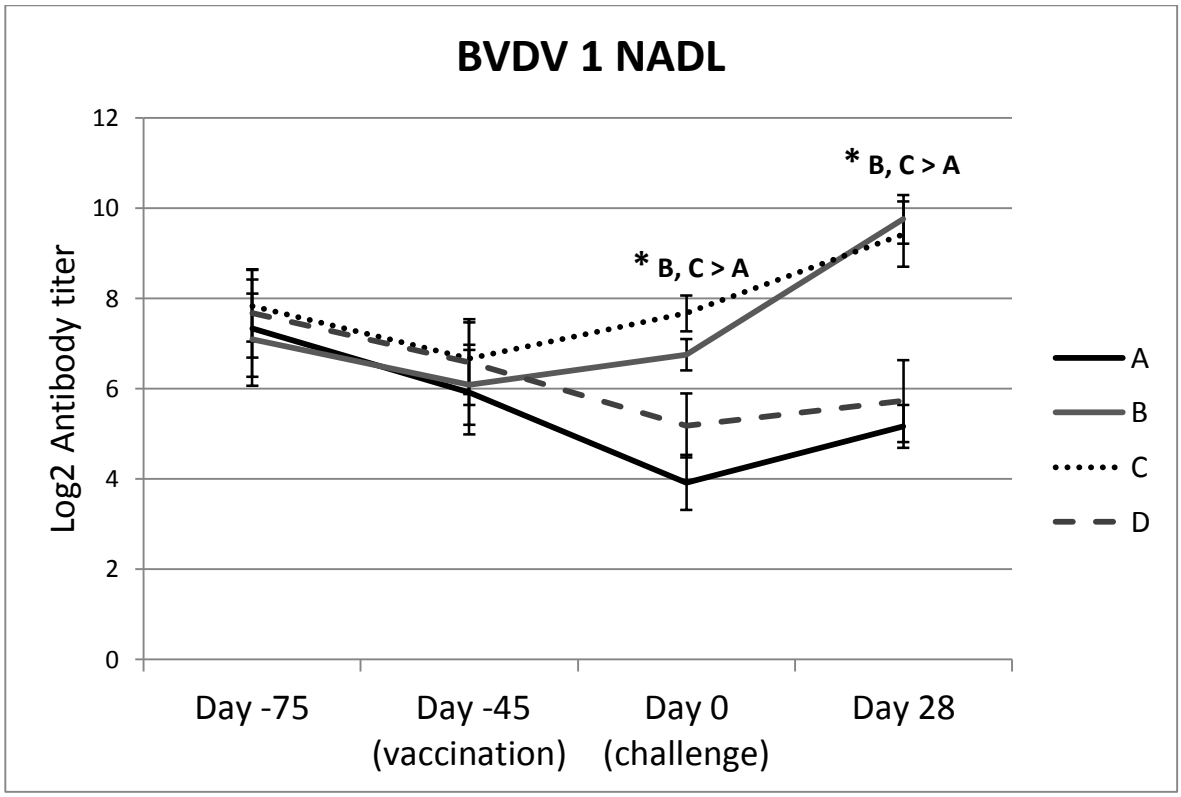


Figure 9. Mean $Log_2$ antibody titers +/- SEM to BVDV 1 NADL in study calves.

Calves from groups B and C had significantly higher mean antibody titers to BVDV 1 compared with the control group A (* = $P < 0.05$).

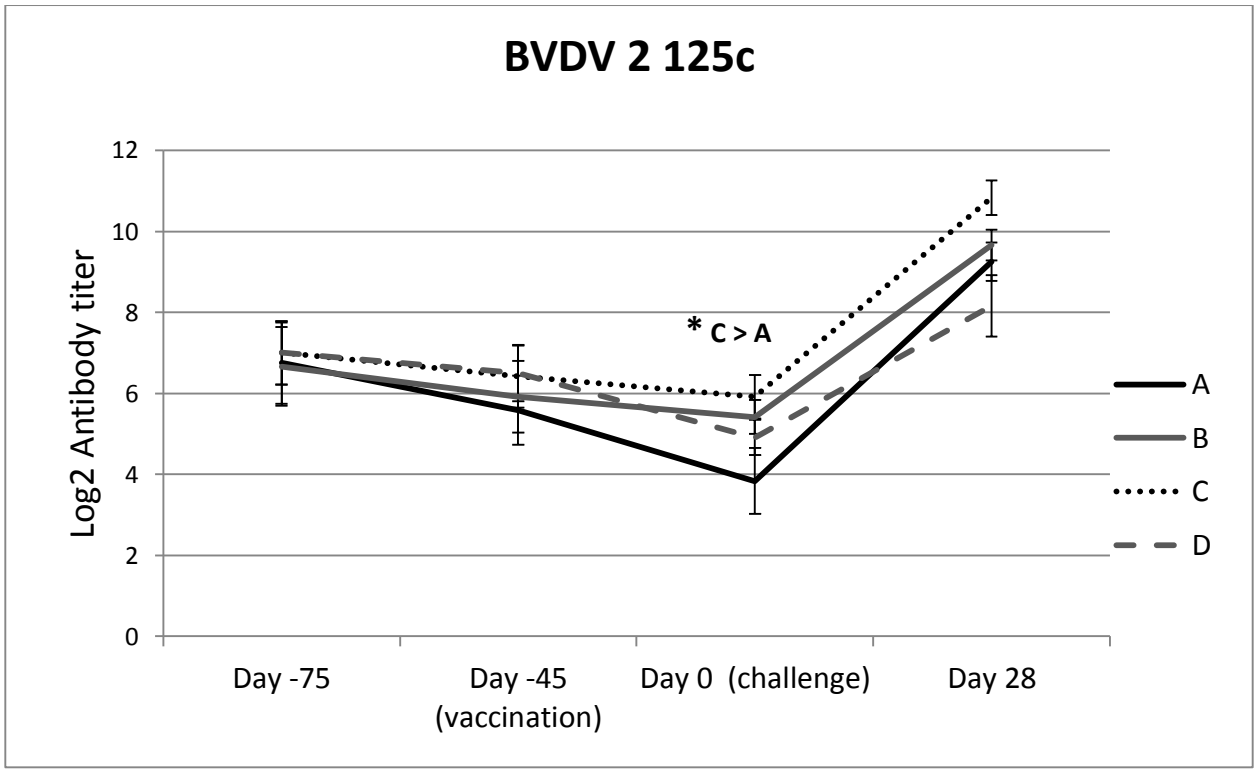


Figure 10. Mean $Log_2$ antibody titers +/- SEM to BVDV 2 125c in study calves. Calves from group C had significantly higher mean antibody titers to BVDV 2 125c compared with the control group A (* = $P < 0.05$).

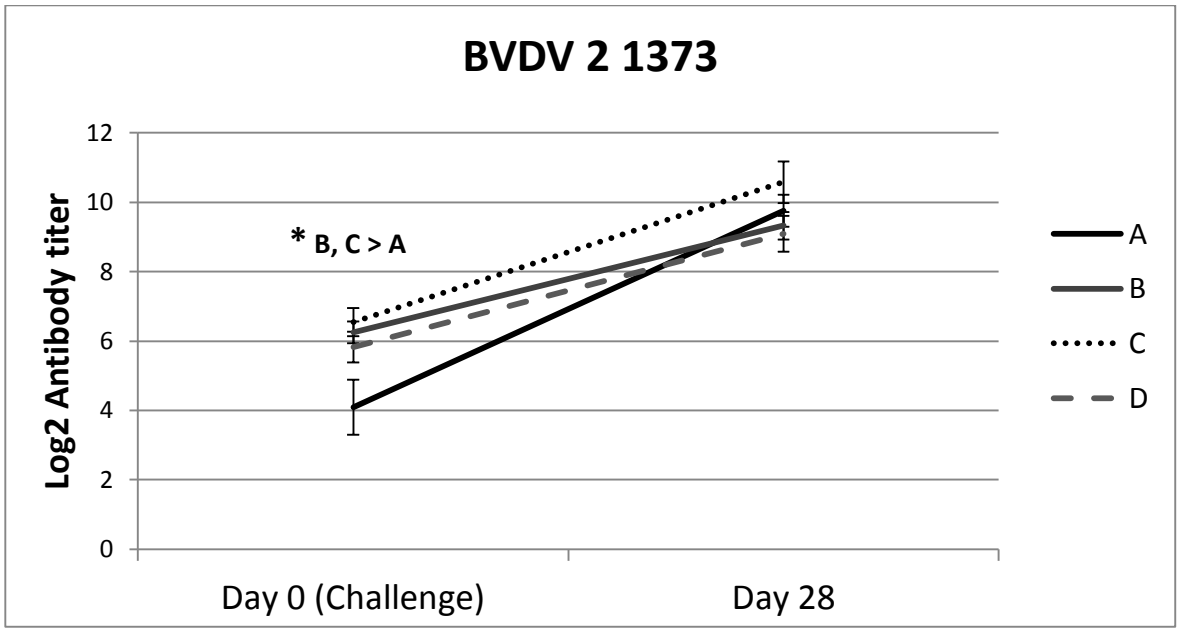


Figure 11. Mean $Log_2$ antibody titers +/- SEM to BVDV 2 1373 in study calves. Calves from groups B and C had significantly higher mean antibody titers to BVDV 2 1373 compared with control group A (* = $P < 0.05$).

*3.2 Virus isolation*

Virus positive samples in serum and/or WBC samples were detected more frequently and for a longer time in the control group compared with groups, B, C, and D (Figures 12). The total proportion of calves that tested positive to BVDV 2 in serum and WBC samples after challenge (days 0 to 28) was higher in the control group (90%) and group D (54.5%) compared with groups B (16%) and C (25%) (Table 4 and Figure 13). A significant effect of group and time (day) was detected on days 6, 8, 10, and 14 after challenge with BVDV 2 1373 ($P = 0.0001$ and $P = 0.0001$, respectively). On day 6 post-challenge, the control group had a higher proportion of calves with positive BVDV samples (58.3%) compared with groups B (8.3%) and C (8.3%) ($P < 0.05$) but not with group D (36.3%). Additionally, at days 8, 10, and 14 post-challenge, a higher proportion

of calves in the control group had BVDV positive samples compared with calves in groups B, C, and D. The proportion of viremic calves (calves with positive serum or WBC samples) that shed virus after challenge (calves whose nasal swab samples tested positive to BVDV by virus isolation) was higher in the control group (72.7%) compared with groups B (0) and C (0) and D (33.3%) ($P < 0.05$).

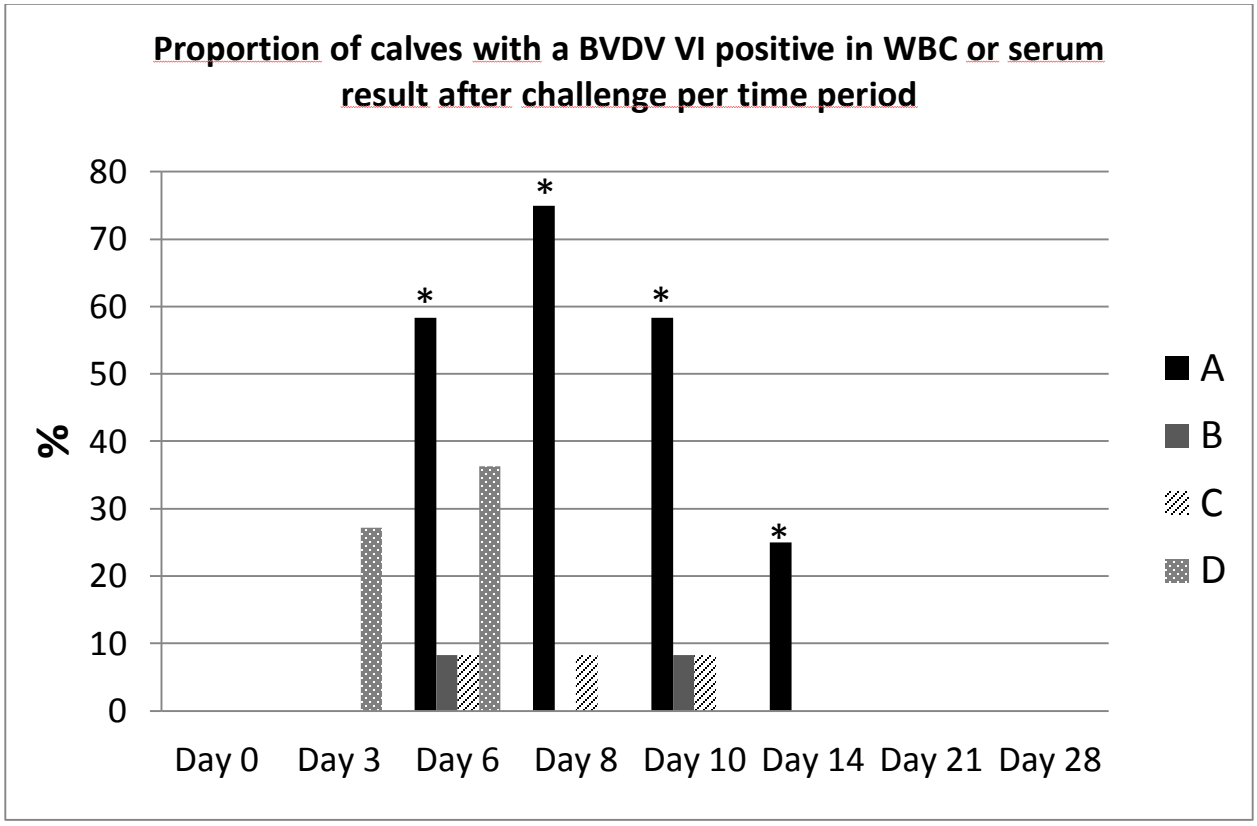


Figure 12. Proportion of calves with a positive virus isolation result in WBC or serum samples at each time point after challenge with BVDV 2 1373. The proportion of vaccinated (B, C and D) and control (A) calves with positive virus isolation WBC or serum samples at each time point (day) after challenge with BVDV 2 1373 was higher

and more frequent in the control group (A) compared with groups B and C and D. (* = P < 0.05).

| Group | Number of viremic¥ calves (%) | Number of viremic calves that shed virus (%) |
|---|---|---|
| **A** | 11 (90)* | 8 (72.7)* |
| **B** | 2 (16.6) | 0 |
| **C** | 3 (25) | 0 |
| **D** | 6 (54.5)* | 2 (33.3) |

Table 4. Total number and proportion of calves (%) that became viremic and shed virus in nasal secretions after challenge (days 0 to 28) with BVDV 2 1373 in each group. ¥, viremic calves = calves with a BVDV positive sample in WBC or serum. A higher proportion of control (A) and group D calves became viremic and shed virus after challenge compared with calves from groups B and C. (* = P < 0.05).

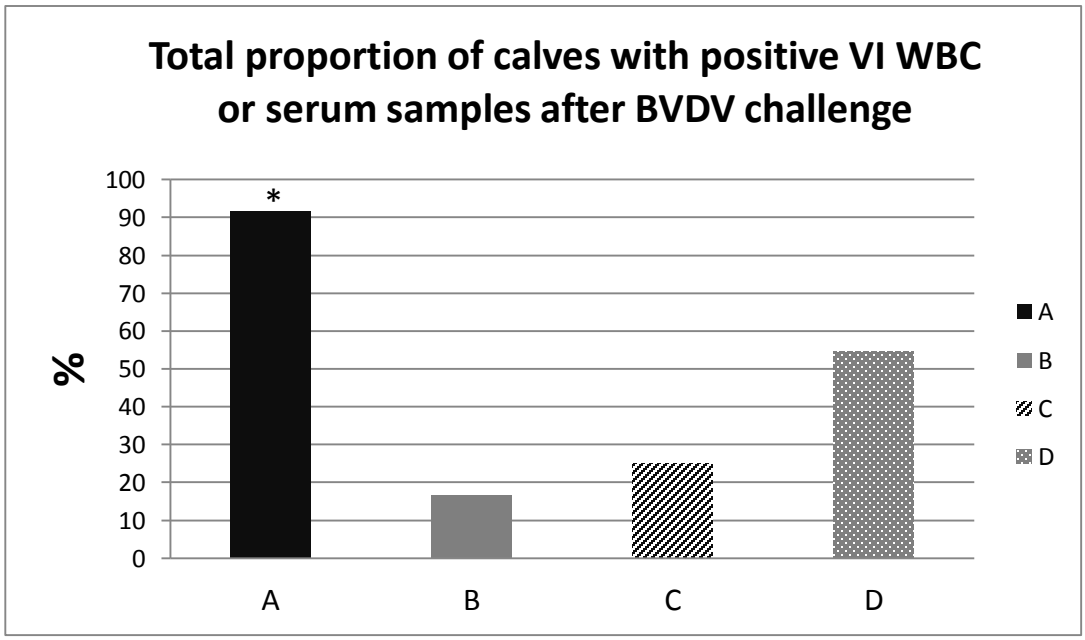

Figure 13. Total proportion of calves that became viremic after challenge (days 0 to 28) with BVDV 2 1373 in each group. ¥, viremic calves = calves with a BVDV positive sample in WBC or serum A higher proportion of control (A) and group D calves became viremic and shed virus after challenge compared with calves from groups B and C. (* = $P < 0.05$).

*3.3 Clinical scores and body weight*

There were no detectable adverse vaccine reactions in any of the calves. One calf in group D was euthanized on the day prior to challenge with BVDV 2 1373 due to rectal prolapse. Vaccinated and control calves demonstrated clinical protection against challenge with virulent BVDV 2 1373 as only one calf in the control group developed mild diarrhea and anorexia on day 14 post-challenge. The proportion of calves with clinical scores of $\geq 2$ for respiratory, diarrhea, and depression parameters was similar between groups ($P > 0.05$). A mild increase in body temperature, nasal secretion, and loose feces was observed in all calves after challenge independent of group designation. A significant effect of time (day) on rectal temperatures was observed ($P < 0.00001$; Figure 14). On day 8, the mean rectal temperatures of all groups were increased compared with other days post-challenge.

The average body weight at weaning (day −45) was not significantly different between groups (A = 206.56 +/− 3.53, B = 207.25 +/− 8.33, C = 214.08 +/− 9.25, D = 188.41 +/− 10.08); however, a significant effect of group and time (day) was detected at day 0 and at the end of the study ($P = 0.0001$ and $P = 0.0001$, respectively). Calves from group B and C had significantly higher mean body weights at both time points compared

with control calves (B = 257.5 +/− 13.14, C = 257.5 +/− 11.87 vs. A = 242.5 +/− 11.08 and B = 345 +/− 15.72, C = 333.75 +/− 12.17 vs. A = 267.91 +/− 12.63, respectively; Figure 15). At the same times (day 0 and end of the trial), the mean body weights of calves from group D were not significantly different compared with control calves (240 +/− 15.25 vs. 242.5 +/− 11.08 and 300.90 +/− 14.7 vs. 267.91 +/− 12.63, respectively) (P = 0.568 and P = 0.262, respectively).

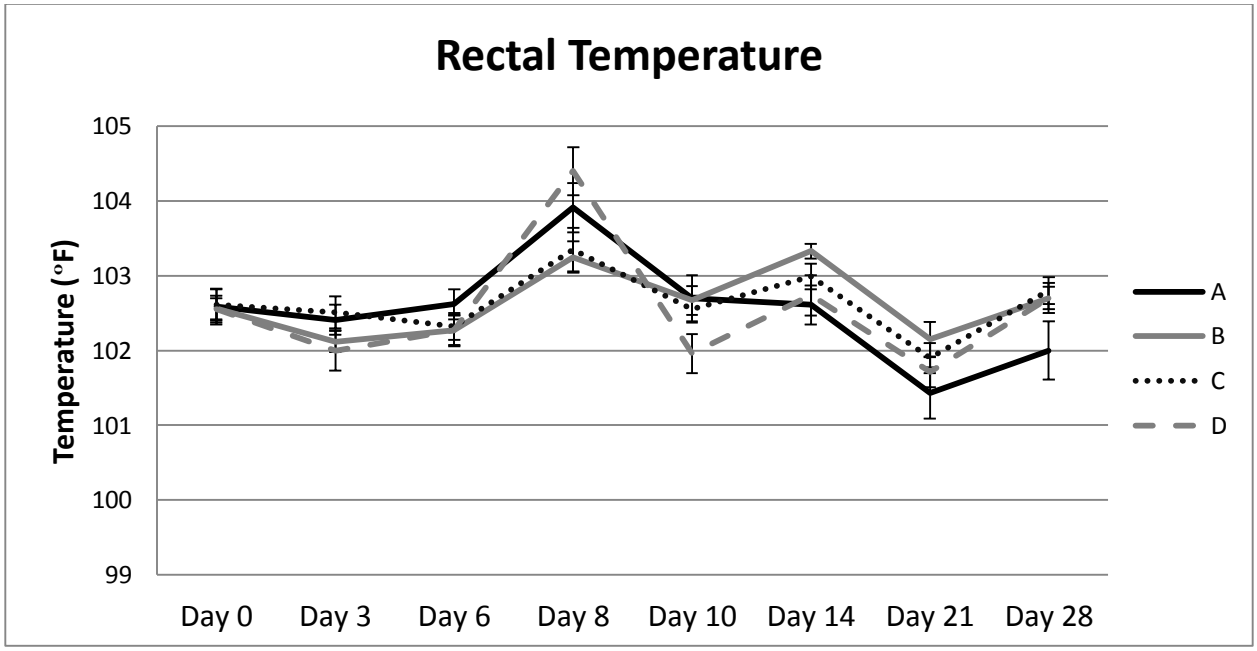


Figure 14. Mean rectal temperatures +/- SEM in study calves after challenge with BVDV 1373. At day 8 after challenge the mean rectal temperatures in all groups were higher; however, mean rectal temperatures were not significantly different among groups.

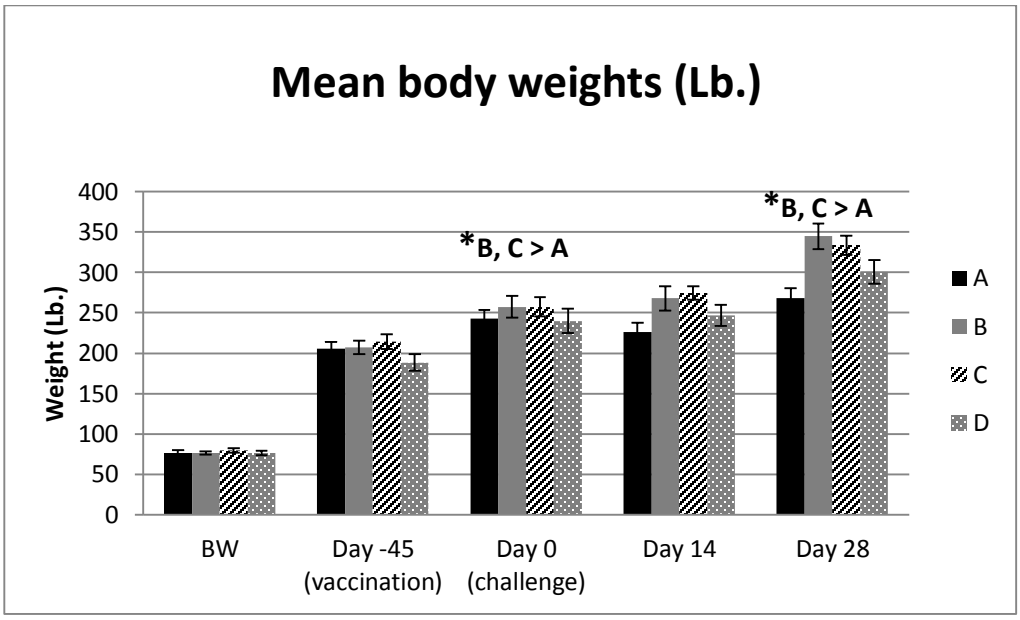


Figure 15. Mean body weights +/- SEM in study calves from birth to the end of the study. At day 0 and at the end of the study calves from group B and C had significantly higher mean body weights at both time points compared with control calves A (* = $P < 0.05$)

*3.4 Hematology*

A significant effect of time (day) but not group was detected in the mean WBC counts, as mean WBC decreased in all groups from day 0 (challenge) until day 6 post-challenge ($P = 0.0001$); however, significant differences were not observed between groups ($P > 0.05$). On day 8 post-challenge, a significant effect of group and time (day) was detected. The mean WBC count from Group D calves was significantly higher compared with control calves ($P = 0.007$). At the same time, the mean WBC from calves in groups B and C were not significantly different compared to control calves (Figure 16). Effects of group and time (day) were not detected in platelet counts at any time point after challenge with BVDV 1373 (Figure 17).

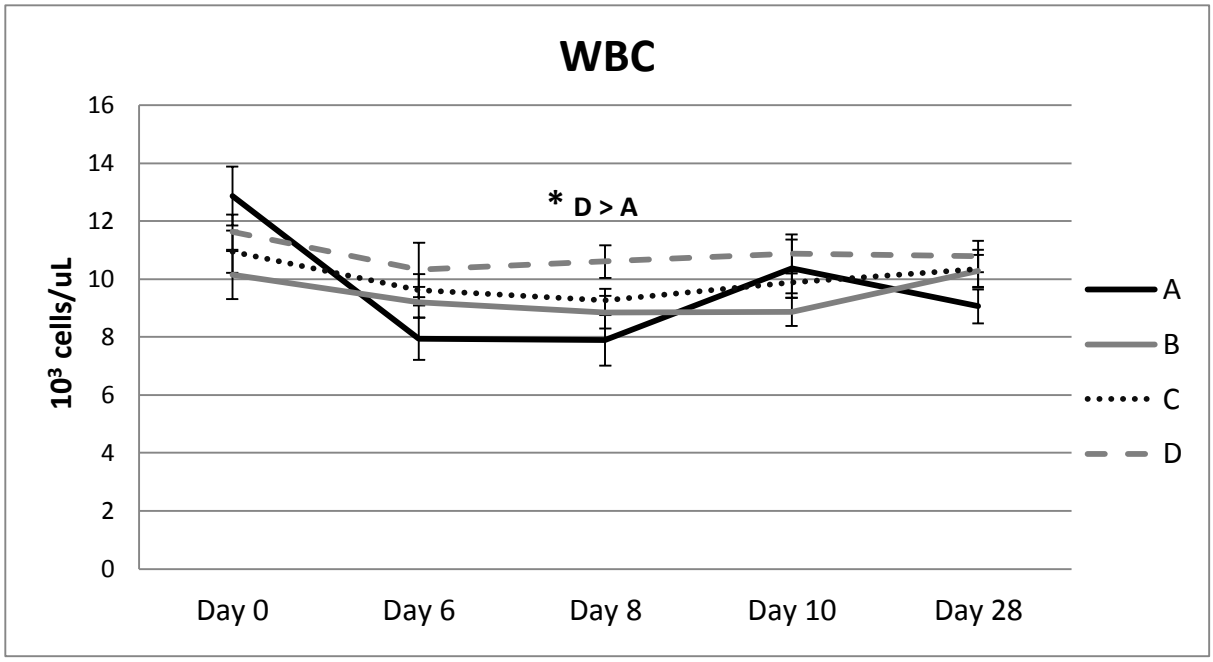


Figure 16. Mean white blood cell count (+/−SEM) after challenge with BVDV 2 1373. Mean WBC between vaccinated (B, C, and D) and control (A) calves after challenge. In all calves mean WBC decreased until day 6 after challenge. At day 8 after challenge calves from group D had higher mean WBC compared with the control group (* P = 0.007).

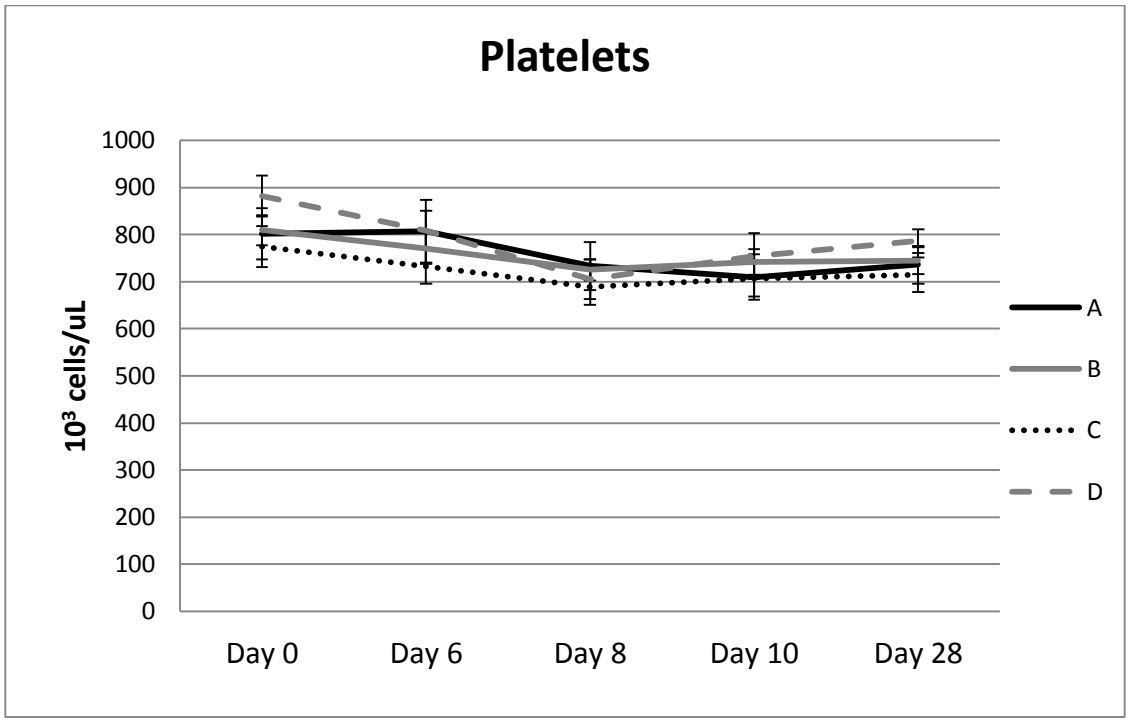


Figure 17. Mean platelet count (+/−SEM) after challenge with BVDV 2 1373. Mean platelet count after challenge with BVDV 2 1373 was not significantly different between vaccinated (B, C, and D) and control calves (A) after challenge with BVDV 1373.

## Discussion

High levels of BVDV-specific antibodies from colostrum or vaccination effectively protect calves against severe clinical disease induced by challenge with virulent BVDV (Cortese et al. 1998c; Platt et al. 2009); however, prevention of viremia and virus shedding are variable in cattle vaccinated with MLV vaccines and subsequently challenged with virulent BVDV (Ridpath et al. 2010b, Stevens et al. 2011; Palomares et al. 2012). In the current study, calves from all groups had similar clinical scores and rectal temperatures, and mortality was not observed after challenge with virulent BVDV

2 1373. This could be associated with protection offered by maternally-derived BVDV antibodies in vaccinated and unvaccinated calves as has been previously reported (Ridpath et al. 2003), or could have resulted from lower virulence of the challenge virus than in previous reports (Stoffregen et al. 2000). Pestiviruses constantly undergo genetic change due to the poor proof-reading capability of the RNA-dependent RNA polymerase, resulting in variability of phenotypic characteristics such as host-cell tropism and virulence (Moya et al. 2000). Repeated passage in cell culture was previously reported to result in attenuation of a BVDV isolate, and may have occurred with the BVDV 1373 used in this study (Deregt et al. 2004).

Interestingly, only calves from group D had an increased white blood cell count compared with control calves at day 8 post-challenge. Prevention of leukopenia is one of several parameters used to evaluate response to vaccination after challenge with BVDV (Ridpath et al. 2003; Platt et al. 2009), and in this case the lack of a decrease in WBC counts observed in group D could have been related to the effects of vaccination or to the presence of high levels of specific maternal antibodies. Despite their ability to reduce clinical disease, maternally-derived BVDV antibodies were not as effective in preventing viremia and viral shedding in calves from the control group as 11/12 (90%) of the calves became viremic and of those 8/11 (72.2%) shed virus after challenge. For this study, we choose VI as our testing method in order to documents clinically relevant shedding of live virus (Dubbey et al. 2014). Prior to challenge, the geometric mean of serum BVDV 2 1373 antibody titers of control calves was significantly lower compared with titers from groups B and C (16.91 vs. 76.10 and 93.05, respectively). Low serum antibody titers prior to challenge with BVDV have been associated with an increased risk of viremia and

clinical disease as demonstrated in previous studies (Cortese et al. 1998c; Ellis et al. 2001). Other reports have indicated that calves with serum maternally-derived BVDV antibody titers < 64 before challenge with virulent BVDV have a higher risk of developing clinical disease and systemic spread of the virus compared with calves with greater antibody titers [Cortese et al. 1998c; Howard et al. 1989).

Antibody response to vaccination with MLV BVDV vaccines of young calves in the presence of maternally-derived BVDV antibodies has produced variable results. Previous studies have demonstrated that 40 to 90-day-old calves with maternally-derived antibody titers ≤ 32 against BVDV 1 and BVDV 2 prior to vaccination seroconvert after vaccination (Menanteau-Horta et al. 1985; Munoz-Zanzi et al. 2002; Fulton et al. 2004; Woolums et al. 2013); additionally, these calves can develop an anamnestic response when a second dose of vaccine is administered [Menanteau-Horta et al. 1985; Woolums et al. 2013). In contrast, similar studies have demonstrated that 3 to 56-day-old calves with BVDV 1 and BVDV 2 maternally-derived antibody titers ≥ 32 prior to vaccination usually do not seroconvert to vaccination and clinical protection against challenge with BVDV is variable (Ellis et al. 2001; Zimmerman et al. 2006; Platt et al. 2009). In our study, a small proportion of calves vaccinated at weaning, at a median age of 72.2 days, seroconverted to BVDV 2 after vaccination. In contrast, 50% of calves from group C seroconverted to BVDV 1 suggesting a greater ability of the vaccine C to overcome maternal interference to BVDV 1. The higher levels of antibodies to BVDV 1 NADL and BVDV 2 1373 before challenge, the lower proportion of viremia, and the absence of viral shedding after challenge observed in calves from groups B and C suggests that vaccination with B and C may have primed B cell responses to increase specific antibody

production or may have delayed the normal decay of maternal BVDV antibodies (Howard et al. 1989; Fulton et al. 2004; Woolums et al. 2013). Additionally, the higher levels of antibodies at challenge could have reduced viremia and prevented viral shedding in calves from the same groups. The reduction of viremia and viral shedding is a highly desirable outcome of vaccination since this could prevent BVDV transmission in operations such as feedlots and stocker units where high numbers of cattle from multiple origins are commingled.

The higher proportion of calves with viremia and virus shedding observed in group D could have been a consequence of the presence of more specific maternal antibodies induced by previous vaccination of the dams with vaccine D. The presence of more specific BVDV antibodies induced by vaccine D on colostra from the dams could have exerted a more efficient blockage of humoral cell responses of calves vaccinated with D. Similar results were detected in a recent study in which lower antibody levels to BVDV 1a, BVDV 1b, and BVDV 2 and a higher proportion of viremia after BVDV 2 challenge were observed in calves 42 days after vaccination with D (Ridpath et al. 2010b). Protection against viremia and virus shedding after experimental challenge with BVDV of calves vaccinated with MLV BVDV vaccines has been commonly associated with activation of T cell mediated immune responses independent of the induction of an adequate antibody response (Endsley et al. 2002; Ridpath et al. 2003; Zimmerman et al. 2006). A previous study reported the depletion of CD4+ lymphocytes in calves acutely infected with BVDV could prolong the duration of viral shedding (Howard et al. 1992). Additionally, in another study, 80% of calves vaccinated at 3 days of age with a MLV BVDV vaccine and challenged with virulent BVDV 2 7–9 months later became viremic

after challenge; however, calves were protected against severe clinical disease in the absence of antibody responses at initial vaccination (Stevens et al. 2011). This indicates that induction of specific T cell memory responses after early vaccination may not always prevent viremia and virus shedding in young calves after challenge with BVDV. In the current study we did not evaluate BVDV-specific T cell responses to vaccination and challenge; however, it is possible that a stronger activation of T cell memory responses in calves vaccinated with D that became viremic could have reduced the duration of viremia to only 2 days; additionally, T cell memory responses in groups B and C could have been associated with the reduced proportion of calves with viremia and nasal shedding.

Previous reports have demonstrated that acute BVDV infection in young calves can result in decreased weight gains and decreased performance (Ridpath et al. 2003; Platt et al. 2009). In the current study, control calves had lower mean body weights at day 0 and at the end of the study compared with calves from groups B and C. Mean body weights of control and group D calves were similar during the study. It is possible that vaccines B and C had a positive effect on weight gain after vaccination as previously reported (Tait et al. 2013). Additionally, the higher frequency of viremia in control and group D calves could have had a negative effect on performance and weight gain. Other study demonstrated that young calves vaccinated with a MLV BVDV vaccine and subsequently challenged with virulent BVDV have higher mean body weights and average daily gains compared with non-vaccinated calves (Platt et al. 2009). The higher mean body weights observed in calves from groups B and C at the end of the study suggest that higher levels of BVDV antibodies before challenge and prevention of viremia could have a positive effect on performance, although this observation would

need further research using larger numbers of experimental subjects. Prevention of weight loss could be a highly desirable outcome of vaccination programs for early weaned beef calves.

## Conclusions

Vaccination of young calves possessing maternally-derived immunity with multivalent MLV vaccines was demonstrated to be beneficial in reducing viremia and virus shedding following BVDV challenge at 45 days after vaccination. Moderate to low levels of maternally-derived BVDV antibodies protected early weaned beef calves against severe clinical disease induced by challenge with virulent BVDV 2. Decay of maternally-derived BVDV antibodies was observed in all groups and just a small proportion of calves seroconverted to BVDV 2 after vaccination; however, the ability of MLV BVDV vaccines to prime B cell responses, induce antibody production, or delay the decay of maternal immunity could result in reduced numbers of viremic calves and prevent virus shedding as was observed in calves from groups B and C in the present study. Reduction of viremia and BVDV shedding could result in decreased BVDV transmission and disease by increasing calf-herd immunity and reducing environmental load of free virus as has been suggested by a previous study (Thurmond et al. 2001). Additionally, MLV BVDV vaccines that reduce viremia after BVDV challenge could have a positive effect on calf performance and would be of most benefit when establishing health programs for early weaned beef calves.

Efficacy of four different multivalent modified-live virus vaccines administered to early weaned beef calves subsequently exposed to cattle persistently infected with bovine viral diarrhea virus and calves acutely infected with bovine herpesvirus-1


## Abstract

The objective of this study was to evaluate the efficacy of four different commercially-available multivalent MLV vaccines to prevent clinical disease, viremia, and virus shedding in early weaned beef calves exposed to six cattle PI with BVDV and eight calves acutely infected with BoHV-1. After weaning (median age = 93.5 days), 54 early weaned steer beef calves were assigned to 1 of 5 vaccination groups: an unvaccinated control group (A), vaccine B, vaccine C, vaccine D, and vaccine E. Forty-five days after vaccination, calves were simultaneously exposed to 6 cattle PI with BVDV and 8 calves acutely infected with BoHV-1. Neutralizing antibody titers for BVDV and BoHV-1 were determined before vaccination and before and after virus exposure. Virus isolation was performed on nasal secretions, serum, and WBC up to 28 days after initial virus exposure. At virus exposure, calves vaccinated with B, C, D, and E had higher neutralizing antibodies to BVDV 1a, BVDV 1b, and BVDV 2. Antibody levels to BoHV-1 were not different between groups during the entire study. Virus isolation and nasal shedding of BVDV were more frequent and persisted longer in calves

in the control group compared to those in B, C, and E groups. Virus isolation and nasal shedding of BoHV-1 were not different among groups. Vaccination of early weaned beef calves resulted in increased BVDV antibody responses and reduced BVDV viremia and nasal shedding. Differences in vaccine efficacy to prevent BVDV viremia and nasal shedding were observed in this study.

M.F. Chamorro, P. H. Walz, T. Passler, R.A Palomares, B.W. Newcomer,J. A. Gard, K.P. Riddell, P.K. Galik, Y. Zhang. "Efficacy of four different multivalent modified-live virus vaccines administered to early weaned beef calves subsequently exposed to cattle persistently infected with bovine viral diarrhea virus and calves acutely infected with bovine herpesvirus-1". *American Journal of Veterinary Research* (manuscript accepted March 18th 2015)

## Introduction

Bovine respiratory disease complex (BRDC) remains the most common disease affecting beef cattle in the United States. Economic losses due to BRDC in the beef cattle industry are estimated around $800 to $900 million annually and include costs associated with mortality, reduced feed efficiency, and antimicrobial treatment (Griffin et al. 1997). Bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BoHV-1) are the viral agents most consistently implicated in contributing to BRDC in young calves. Both viruses are capable of disrupting the mucosa of the upper respiratory tract, suppressing innate and adaptive immune responses, and acting in synergism with other pathogens

such as *Mannheimia haemolytica* and *Mycoplasma bovis* to cause severe disease (Jones et al. 2010; Ridpath 2010).

Vaccination of young calves against viral respiratory pathogens such as BVDV 1, BVDV 2, and BoHV-1 is considered a key strategy of herd health programs to minimize mortality and economic losses associated with BRDC (Step et al. 2009). At feedlot entry, calves possessing greater levels of serum antibodies to BVDV 1, BVDV 2, and BoHV-1 obtained from maternal colostrum or vaccination are less likely to develop clinical disease associated with BRDC (Moerman et al. 1994; Martin et al. 1999; Fulton et al. 2002); however, high levels of maternally-derived antibodies may negatively influence humoral responses to vaccination against respiratory pathogens in calves; in contrast, decay of colostral immunity may decrease protection against field infections (Fulton et al. 2004). Several studies have demonstrated that the outcome of calf vaccination could vary depending on the age of the calf and the level of maternally-derived antibodies to BVDV and BoHV-1 (Menanteau-Horta et al. 1985; Ridpath et al. 2003; Peters et al. 2004; Fulton et al. 2004). Vaccination of young calves possessing maternal immunity with killed virus (KV) or modified-live virus (MLV) vaccines can result in different outcomes including complete blockage of antibody responses, increased persistency of maternal antibodies, seroconversion, and anamnestic humoral responses and clinical protection at virus challenge or second vaccination (Menanteau-Horta et al. 1985; Ellis et al. 2001; Ridpath et al. 2003; Peters et al. 2004; Fulton et al. 2004). Clinical protection of calves that are vaccinated in the presence of maternal antibodies and then experimentally challenged with BVDV and BoHV-1 is associated with activation of naïve B and T lymphocytes that induce virus-specific cell mediated and humoral immune responses

after vaccination (Brar et al. 1978; Ellis et al. 1996; Ellis et al. 2001; Ridpath et al. 2003; Endsley et al. 2004).

In addition to protecting against clinical disease, vaccination of young calves against respiratory viruses such as BVDV and BoHV-1 must reduce virus transmission. A previous study demonstrated that vaccination of a portion of the calf herd with KV and MLV vaccines containing BVDV increased the calf-herd immunity and decreased overall BVDV transmission by 48% (Thurmond et al. 2001). Therefore vaccination of early weaned beef calves prior to shipping to stocker units or feedlots could reduce the overall incidence of BRDC after arrival as a consequence of reduction of BVDV and BoHV-1 shedding and transmission. Several studies have demonstrated that a single dose of a multivalent MLV vaccine containing BVDV and BoHV-1 protects calves possessing maternally-derived immunity against acute clinical disease caused by these viruses (Ellis et al. 2005; Zimmerman et al. 2006; Ellis et al. 2009; Step et al. 2009); however, the efficacy of different multivalent MLV vaccines containing BVDV 1, BVDV 2, and BoHV-1 to prevent virus shedding following challenge has not been evaluated. The objective of this study was to compare the efficacy of four different commercially available, multivalent, MLV vaccines containing BVDV and BoHV-1 in preventing clinical disease, viremia, and virus shedding when administered to early weaned beef calves subsequently exposed to cattle persistently infected with BVDV and calves acutely infected with BoHV-1 forty-five days after vaccination.

## Materials and methods

*2.1 Animals*

Fifty-four clinically normal crossbred beef steer calves were utilized in this study. Calves were born and raised at the Upper Coastal Plain Agricultural Research Center at Winfield AL, a 200-cow cow-calf operation unit from the College of Agriculture and Animal Sciences of Auburn University. Calves eligible for the study were all males born between September 1st and October 1st 2013 to cows that had received a single dose of a MLV BVDV and BoHV-1 vaccine [a] prior to breeding. At birth, calves were identified by ear tattoo and ear tag. All calves remained with their dams and consumption of colostrum occurred under natural conditions in the pasture. A blood sample for detection of neutralizing antibodies to BVDV 1, BVDV 2, and BoHV-1 was collected from all calves at day -75 of the study at a median calf age of 40.3 days.

*2.2 Experimental design*

The study was designed as a randomized control trial. Sample size was calculated on the basis of the expected geometric means (i.e. 15 vs. 250) of antibody titers for each vaccinated group, the standard deviations (i.e. 1.35) of the means, and a statistical power of 99%. All calves were early weaned on day -45, which corresponded with a median calf age of 95 days (range: 60-128 days). Due to high variability on initial serum neutralizing antibodies to BVDV 1a, calves were stratified by initial maternally-derived BVDV 1a antibody titers and randomly assigned to 1 of 5 treatment groups using a random number generator function [b]. The stratification by initial levels of BVDV 1a antibodies ensured that each group received similar numbers of calves with different levels of maternally-derived BVDV 1a antibodies. Five individual plastic bags labelled with the letters A, B,

C, D, and E, respectively, and each filled with 11 2-mL syringes containing their respective vaccine were made the day of vaccination so personnel administering vaccines were blinded to treatment allocation. All calves underwent abrupt weaning on day -45 of the study and were vaccinated once according to their assigned treatment group A (n=11), B (n=11), C (n=10), D (n=11), or E (n=11). Following vaccination, calves were separated in isolated pastures to prevent transmission of vaccine strains between groups for 28. After this time all calves were regrouped in a single pasture. Daily observation of the calves was performed to evaluate for adverse vaccine reactions by farm personal unrelated with the study that were blinded to treatment allocations. Forty-four days after weaning, calves were transported 192 miles to the North Auburn BVDV Isolation Unit located in Auburn, AL. Upon arrival, calves were given access to fresh water and hay. On the next day (day 0), all calves were commingled in the same pen with 6 PI cattle with different strains of BVDV (2 cattle each PI with BVDV 1a, 1b and 2) and 8 calves acutely infected with BoHV-1 for 28 days. Clinical evaluation and sampling of calves was performed until day 28 after initial virus challenge exposure to BVDV and BoHV-1. The primary outcomes of this study were overall mortality rate, presentation of clinical disease (clinical disease scoring), and overall rates of viremia and viral shedding among experimental calves after challenge exposure with BVDV and BoHV-1. The secondary outcomes were antibody responses as a surrogate measure of protection, total white blood cell counts, and weight gains. All research protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University (PRN # 2013-2385).

*2.3 Vaccines*

All vaccines used were USDA-licensed stock material approved for commercial sale. A single dose of vaccine was administered to calves according to label directions at weaning (day -45). Group A was the control group and received 2 mL of 0.9% phosphate buffered saline subcutaneously (SC) once. Group B received 2 mL of vaccine B[c] SC once, group C received 2 mL of vaccine C[d] SC once, group D received 2 mL of vaccine D[a] SC once, and group E received 2 mL of vaccine E[e] SC once. All vaccines were multivalent containing modified-live BVDV 1, BVDV 2, BoHV-1, parainfluenza virus 3 (PI3V), and bovine respiratory syncytial virus (BRSV).

*2.4 BVDV challenge exposure*

From day 0 to day 28, 6 cattle PI with different strains of BVDV were commingled in the same pen with all the experimental calves where they shared feed and water sources. Two cattle were PI with BVDV 1a, 2 cattle were PI with BVDV 1b, and 2 cattle were PI with BVDV 2. For each animal, persistent BVDV status was confirmed by virus isolation and titration (10-fold dilution) from serum samples and nasal swab specimens on day 0. Previous to commingling on day -8, the 6 PI cattle were vaccinated intranasally once with a MLV vaccine that contained BoHV-1, PI3V, and BRSV.[f]

*2.5 BoHV-1 challenge exposure*

On days -7 and -5, eight 4-month-old steer calves that were seronegative to BoHV-1 were inoculated with $3 \times 10^7$ $CCID_{50}$ of BoHV-1 (Colorado strain)[g] intravenously. Four calves were inoculated on day -7 and the other four were inoculated on day -5 to assure that inoculated calves were in the course of acute BoHV-1 infection at

initiation of commingling. These calves were then commingled during days 0 through 28 with all the experimental calves in the same pen where they shared feed and water sources. For each calf, the BoHV-1 titer was determined by virus isolation from serum samples and nasal swab specimens on days 0, 3, 7 and 14.

*2.6 Sample and data collection*

Clinical evaluation of calves was performed on days 0, 3, 6-10, 14, 21, and 28. Individual rectal temperatures, serum, whole blood, and deep nasal swab samples were collected on those same days for virus isolation, qPCR, virus neutralization, and complete white cell count (CBC) testing. Collection of samples was performed by the principal researcher who was not blinded to treatment allocation; however, samples from each calf were labelled such that treatment allocation remained masked from personnel processing the samples at the laboratory. The laboratory personnel that processed and analysed samples did not participate in treatment allocation designation nor in collection of samples during the course of the study.

During sampling days and prior to handling, each calf was evaluated by a veterinarian who was not aware of treatment allocation and who was only responsible for score each calf for signs of abnormal respiration, diarrhea, and depression using a scale of 0 to 3. The absence of clinical signs scored was as 0 and the most severe clinical signs was scored as previously described (Givens et al. 2012). Briefly, an abnormal respiratory score was given if an animal presented with abnormal nasal discharge. Nasal discharges were judged as being serous, mucous, or mucopurulent. Diarrhea scores were judged as being normal feces, pasty feces, runny feces, or severe diarrhea with or without blood.

Depression scores ranged from no depression, mild depression, moderate depression, or severe depression. In addition to visual examination, individual body weights were obtained on day -45 (vaccination/weaning day), day 0 (initial BVDV and BoHV-1 virus challenge exposure), and days 14, 28, and 56 using a portable livestock electric scale[h] that was validated prior to and after each weighing.

*2.7 Virus titration*

Virus titration was performed on serum and nasal swab specimens from the 6 cattle PI with BVDV on days 0 and 28 and from BoHV-1 acutely infected calves on days 0, 3, 7, and 14 as previously described (Givens et al. 2012). Briefly, serial 10-fold dilutions (1:10 to 1:10,000,000) of serum and nasal swab specimens were performed in triplicate; the statistical method of Reed and Muench (1938) was used to determine the quantity of BVDV or BoHV-1. Each 10 μL dilution of serum and nasal swab specimen was assayed by adding to 90 μL of media to 3 wells of a 96-well plate, which was subsequently seeded with approximately 2,500 MDBK cells in 50 μL of MEM. To determine the concentrations of BVDV, plates were incubated for 3 days at 37°C in a humidified atmosphere of 5% CO2 and air. Subsequently, an immunoperoxidase monolayer assay was used as a labeling technique to confirm the presence of BVDV in the cultured MDBK cells (Givens et al. 2012). To determine the concentrations of BoHV-1, plates that contained samples from the 8 calves with acute BoHV-1 infection were incubated for 5 days at 38.5°C in a humidified atmosphere of 5% CO2 and air, and wells were examined daily for the characteristic cytopathic effect associated with BoHV-1.

*2.8 Virus isolation (VI)*

Whole blood and serum samples collected on days 0, 3, 6-10, 14, 21, and 28 after virus challenge exposure were used for BVDV by VI. Serum and deep nasal swab samples collected on the same days were used for BoHV-1 detection by VI as previously described (Walz et al. 2008; Givens et al. 2012). Briefly for BVDV, isolated WBC samples (buffy coat) and serum were subjected to co-cultivation in 24-well plates containing monolayers of MDBK cells. The 24-well plates were incubated for 4 days at 37°C and 5% $CO_2$. Following cultivation, all plates were frozen at -80°C and thawed, and 10 µl of the sample was inoculated in triplicate into wells on 96-well microtiter plates followed by MDBK cells in culture medium. After 72 hours of incubation at 37°C and 5% $CO_2$, all wells were stained to detect BVDV using the immunoperoxidase monolayer assay as previously described (Walz et al. 2008). For BoHV-1, 24-well plates containing nasal swab samples were incubated for 96 hours and examined daily for characteristic cytopathic effect of BoHV-1. After 4 days, the plates were frozen and thawed and qPCR was performed to detect BoHV-1 DNA.

*2.9 Real-time qPCR for BVDV and BoHV-1 on nasal swab samples*

Because of a high number of nasal swab samples exhibiting cytopathic effect in the 24-well plates, all nasal swab samples were subjected to qPCR testing for BVDV and BoHV-1. A membrane kit[i] was used to extract viral RNA or DNA from nasal swab specimens on days 0, 3, 6-10, 14, 21, and 28 according to the manufacturer's instructions. A SYBR Green-based real-time PCR protocols were developed to detect BVDV and BoHV-1 as previously described (Herlekar et al. 2013). The optimal PCR conditions

were determined to be the lowest concentration of the standard template required to amplify the correct gene fragment corresponding to each virus. Forward and reverse primers were used for both viruses, BVDV-F 5' – TAGCCATGCCCTTAGTAGGAC – 3', BVDV-R 5' – GACGACTACCCTGTACTCAGG – 3'; BoHV-1-F 5' – GTAAGGGTATATTATTGATTGC – 3', BoHV-1-R 5'- GACAGTGAGTATGAGGAC -3', respectively. For BVDV, the reaction mixture in each tube contained 5µL of RNA template, 10 µL of iTaq SYBER Green master mix[j], 0.25 µL of reverse transcriptase[j], 0.3 µ*M* (0.3 µL) each of forward and reverse primers, and sterile PCR water to make up the total reaction volume of 20 µL. The PCR protocol for detection of BVDV was 50ºC for 10 min for 1 cycle, 95 ºC for 1 min for 1 cycle, followed by 95 ºC for 10s and 56.5 ºC for 30s for 39 cycles. A positive control was maintained using the BVDV standard template. No-template control was maintained using PCR-grade water instead of the standard template. An additional control without reverse transcriptase was maintained for the BVDV PCR assay. For BoHV-1, the reaction mixture contained 5µL of DNA template, 10 µL of Ssoadvanced universal SYBR Green supermix[k], 0.5 µ*M* (0.5 µL) each of forward and reverse primers for BoHV-1, and sterile PCR-grade water for a total volume of 20 µL. The PCR protocol for detection of BoHV-1 was 1 cycle at 98ºC for 3 min followed by 95 ºC for 15s and 60 ºC for 3s for 35 cycles. The standard DNA template served as positive control. The non-template control served as negative control, which contained PCR water instead of DNA template. All the reactions were subjected to real-time PCR using a real-time PCR detection system .[l] The qPCR yielded the expected products for BoHV-1 (128 bp) and BVDV (96 bp).

*2.10 Sequencing of BVDV isolates*

A two-round rapid-cycle RT-nPCR assay was performed to confirm BVDV in WBC samples for further genotyping procedures. Positive and negative controls were included in the RNA extraction step and RT-nPCR assay as previously described (Givens et al. 2012). All steps of the RT-nPCR were performed in a single-tube reaction. In the first round, the outer primers, BVD 100 (5′-GGCTAGCCATGCCCTTAG-3′) and HCV 368 (5′- CCATGTGCCATGTACAG-3′) amplified a 290 base pair sequence of the 5′ untranslated region of the viral genome. In the second round, the inner primers BVD 180 (5′-CCTGAGTACAGGGDAGTCGTCA-3′) and HCV 368 amplified a 213 base pair sequence within the first amplicon. After completion of the PCR cycle, 10 µl of the RT-nPCR products were separated by 1.5% agarose gel electrophoresis. Visualization of the RT-nPCR product was performed by Gel Red staining and ultraviolet transillumination. When a WBC sample was confirmed positive for BVDV, viral RNA within the sample was amplified in triplicate. The resulting RT-nPCR products were then purified by use of a silica gel–based membrane kit[m] and sequenced via automated dye terminator nucleotide sequencing by use of both the 5′ (BVD 180) and 3′ (HCV 368) primers. Consensus sequences were determined with computer software[n] and compared with the BVDV sequences obtained from the 6 PI cattle used for the BVDV challenge exposure to determine the genotype of the infecting virus.

*2.11 Virus neutralization*

The standard virus neutralization microtiter assay was used to detect antibodies to BVDV and BoHV-1 in serum collected on days -75, -45, 0 (prior to virus challenge

exposure), and 28 (Walz et al. 2008). Sera were tested for neutralizing antibodies against BVDV types 1a (BVDV strain NADL), 1b (BVDV strain TGAC), and 2 (BVDV strain 125c) and BoHV-1 (Colorado strain). For the BVDV neutralization assays, following heat inactivation of serum samples at 56°C for 30 minutes, serial 2-fold dilutions were made in 50 μL of culture medium. For each dilution, 3 wells of a 96-well plate were inoculated with an equal volume (50 μL) of culture medium containing 100-500 $CCID_{50}$ of the test strain. After inoculation, the plate was incubated at 38.5°C in a humidified atmosphere of 5% $CO_2$ and room air for 1 hour. Then, 2.5 x $10^3$ MDBK cells in 50 μL of culture medium were added to each well. Plates were incubated for 72 hours and evaluated visually for cytopathic effect (Walz et al. 2008). For the BoHV-1 neutralization assay, after each well was inoculated with the respective serum dilution, plates were incubated for 96 hours at 38.5°C in a humidified atmosphere of 5% CO2 and air, and then each well was examined for the characteristic cytopathic effect of BoHV-1. The geometric mean of antibody titers was calculated from the endpoint $Log_2$ titers of the animals in each group.

*2.12 Hematology*

Whole blood samples were submitted for leukocyte and platelet analysis on day 0 prior to BVDV and BoHV-1 challenge and on days 8, and 14 during the period of virus exposure. The total leukocyte count for each sample was determined by use of an automatic cell counter.[o]

*2.13 Statistical analysis*

Data were analyzed with a statistical software package. [p] A logarithmic base 2 transformation was applied to VN values before calculation and comparison of the GMs. Back-transformed GMs for each viral strain tested were calculated for each group at days -75, -45, 0 (prior to virus exposure) and 28 days after BVDV and BoHV-1 challenge exposure.

To detect differences in continuous numeric variables such as $Log_2$ transformed antibody levels, rectal temperatures, white blood cell counts, platelet counts, and body weights over time, a mixed generalized linear model for repeated measurements with fixed effects for treatment, day (time), their interaction, and random effects for animal and residual was performed.

An ANOVA was performed to compare means of $Log_2$ transformed antibody levels, rectal temperatures, white blood cell counts, platelet counts, and body weights among the treatment groups by the use of a generalized linear model. To adjust for multiple comparisons, treatment comparisons and individual days were only made if the overall treatment effect or the interaction between treatment and day was significant. The Dunnett test was used to detect differences between vaccinated groups and the control group. A multivariate repeated measures ANOVA was used to evaluate ordinal categorical data from clinical scores after virus exposure.

To detect differences from nominal categorical data such as virus isolation results from serum and nasal swab specimens a logistic regression procedure (Proc Logistic) was performed and results were compared by use of a $\chi^2$ test. A logistic regression and $\chi^2$ test was also used to analyze numerical continuous variables such as proportions of

seroconversion, RT-nPCR, and qPCR positive results. For all analyses, values of $P < 0.05$ were considered significant.

## Results

### *3.1 Virus challenge exposure with BVDV PI cattle and BoHV-1 infected calves*

Bovine viral diarrhea virus was detected in serum samples and nasal swab specimens obtained from all 6 PI cattle on days 0 (the first day of BVDV challenge exposure) and 28 (last day of BVDV challenge exposure). The BVDV titers in serum ranged from $1.1 \times 10^3$ to $6.2 \times 10^4$ CCID50/mL, and those in nasal swab specimens ranged from $6.2 \times 10^3$ to $1.1 \times 10^6$ CCID50/mL. The PI cattle had no clinical signs of BVDV or BoHV-1 infection during the virus challenge exposure period. Transmission of BVDV among the commingled cattle was confirmed by isolation of BVDV in serum, WBC, and nasal swab specimens from experimental calves. Bovine herpesvirus 1 was detected on days 3, 6, 7 and 14 by qPCR in nasal swab specimens in 7 of the 8 calves intravenously inoculated with BoHV-1. Titers of BoHV-1 were not detected in serum samples at any time point from inoculated calves. Transmission of BoHV-1 among the commingled cattle was confirmed by the presence of BoHV-1 DNA in nasal secretions of experimental calves.

### *3.2 Clinical scores and body weights*

There were no detectable adverse vaccine reactions in any of the experimental calves. Vaccinated and control calves demonstrated clinical protection against

simultaneous natural exposure to BVDV and BoHV-1 as none of calves in the vaccinated or control groups developed severe clinical signs of disease or mortality. The mean clinical scores during BVDV and BoHV-1 challenge exposure period (Days 0 to 28) were not different between treatment groups. The total number of calves that presented abnormal clinical scores (score of $\geq 1$) at least once for respiratory, diarrhea, and depression parameters during the virus challenge exposure period was similar between groups ($P > 0.05$, Table 5). The mean rectal temperature was not significantly different among treatment groups after challenge exposure to BVDV and BoHV-1 ($P > 0.05$, Figure 2). A significant effect of time in mean rectal temperatures ($^0$F) was detected in all groups on day 7 when all calves presented a higher mean rectal temperature (group A: 103; group B: 103.5; group C: 103.5; group D: 103.1; group E: 102.8) compared to mean rectal temperatures on day 0 (group A: 102.3; group B: 102.9; group C: 102.6; group D: 102.4; group E: 102.4) and subsequent days post virus exposure ($P < 0.00001$; Figure 18). The average body weight at weaning (day -45), at day 0 (first day of challenge exposure), at day 28 (last day of challenge exposure), and at day 56 was not significantly different among treatment groups ($P > 0.05$); however, mean body weights increased significantly over time in all groups (Figure 19).

| Group | Respiratory score | Diarrhea score | Depression score |
|---|---|---|---|
| **A** | 11/11 (100) [a] | 7/11 (63.6) [a] | 7/11 (63.6) [a] |
| **B** | 10/11 (90.09) [a] | 6/11 (54.5) [a] | 7/11 (63.6) [a] |
| **C** | 9/10 (90) [a] | 7/10 (70) [a] | 6/11 (60) [a] |
| **D** | 10/11 (90.09) [a] | 8/11 (72.27) [a] | 7/11 (63.6) [a] |
| **E** | 8/11 (72.27) [a] | 7/11 (63.6) [a] | 6/11 (54.5) [a] |

Table 5. Number and proportion (%) of calves that demonstrated abnormal clinical scores (≥ 1) for respiratory, diarrhea, and depression signs at least once during the 28 days of the challenge exposure with BVDV and BoHV-1. Groups not connected by the same letter within each clinical sign are significantly different ($P < 0.05$).

absence (solid line) or presence (dashed line) of DB772. The mutations associated with each isolate are shown in the inset.

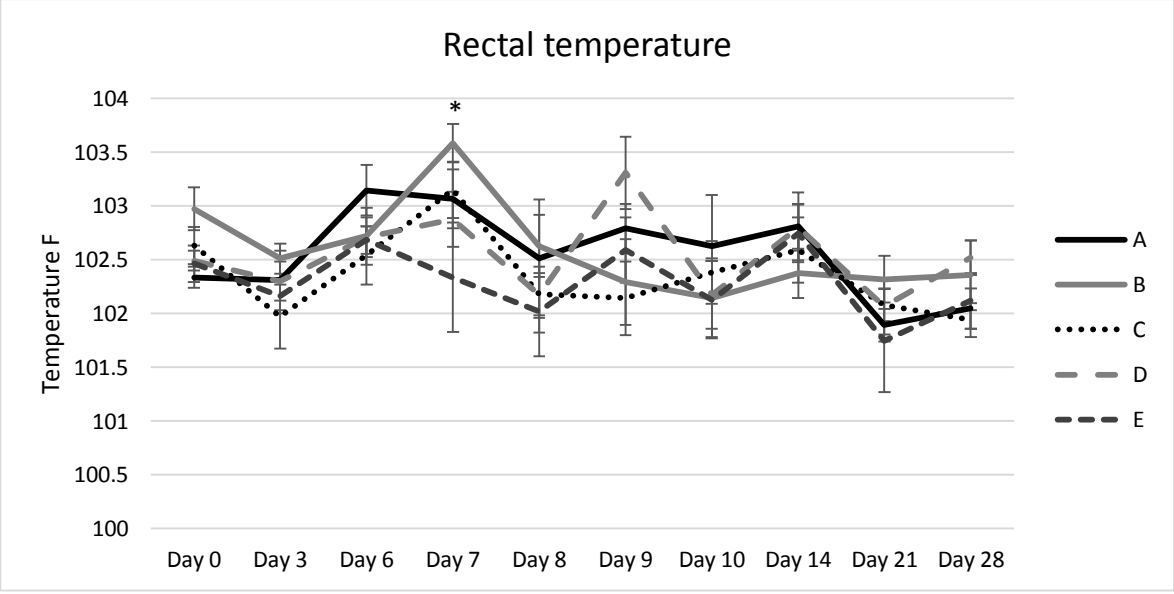

Figure 18. Mean rectal temperatures ($F^0$) +/- SEM among study calves following challenge exposure with BVDV and BoHV-1. The mean rectal temperatures were not different between groups. At day 7 after virus challenge exposure a significant effect of time was detected as all groups had an increased mean rectal temperature compared with other time periods (* $P < 0.00001$).

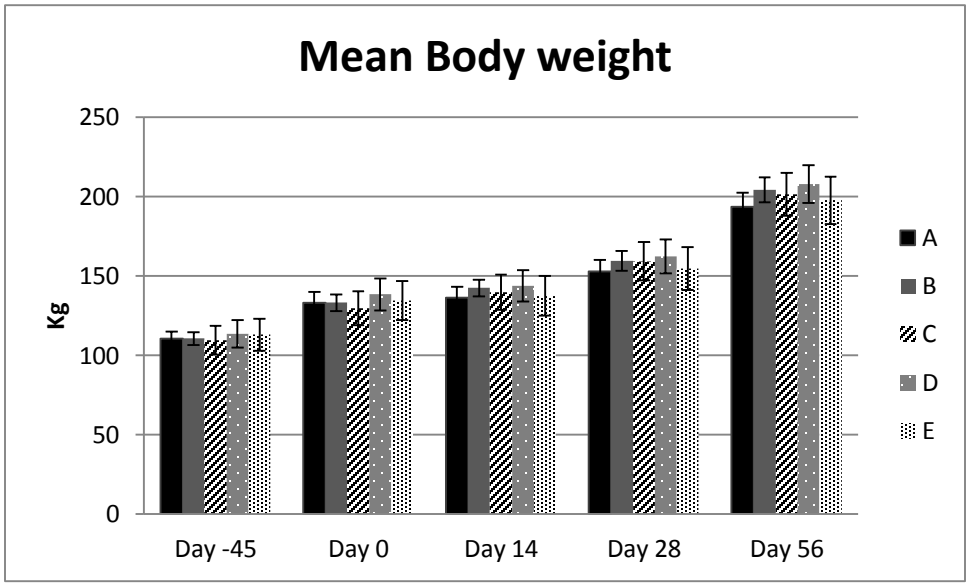


Figure 19. Mean body weights (Kg.) +/- SEM of study calves during the study period. The mean body weights of calves during the study period were similar between groups; A Significant effect of time was detected as all calves increased their mean body weight over time ($P < 0.05$)

*3.3 Virus isolation and PCR assays*

All study calves were negative for BVDV and BoHV-1 by VI at day 0 prior to virus challenge exposure. Positive results of BVDV in WBC or serum samples were obtained from day 3 to 14 after challenge in 9 calves from the control group, on day 6 in

one calf from group B, on day 14 in 2 calves from group C, and from day 6 to 10 in 4 calves from group D. Positive results of BVDV in WBC and serum were not detected in calves from group E after challenge. During the first 2 weeks of virus exposure (days 6,7, 8, 9, 10 and 14), a higher proportion of calves from the control group had BVDV positive serum or WBC samples compared with calves from groups B, C, and E (Table 6). On days 6, 7, 8, 9, 10 and 14, the number of calves with viremia in the unvaccinated control group (n=6) was significantly higher ($P < 0.05$) than the number of calves with viremia in groups B (n = 1, $P$=0.02) C (n=0, $P$= 0.005) and E (n=0, $P$= 0.005 ) but was not significantly different from the number of calves with viremia in group D on days 6, 7, 8, and 9 post exposure (Figure 20).

In general, the cumulative number of viremic calves (calves with one or more BVDV-positive serum and WBC samples) during the experimental period was significantly higher in group A (9 out of 11 $P$=0.0006) than that for groups B, C, and E. Significant differences were not found for the cumulative number of viremic calves between groups A and D. After day 14 of BVDV exposure, viremia was not detected in any of the study calves.

The total number of calves that shed BVDV (BVDV qPCR-positive in nasal swab samples) during the experimental period was higher in the control group (5 out of 11) compared with calves in groups C (1 out of 10), and E (no shedding) ($P$= 0.07 and $P$= 0.01), respectively (Table 6); However, there was no difference in the proportion of calves that shed BVDV in groups A, B and D (5, 2, and 3 calves for groups A, B and D, respectively). Calves from the control group shed BVDV from day 6 through day 21 after virus challenge exposure. The 2 calves that shed BVDV in group B shed on days 9 and

28, respectively. The only calf that shed BVDV in group C did so on day 8, and calves the three calves from group D shed BVDV on days 8, 9, and 21, respectively. Five out of five calves that shed BVDV in the control group were viremic after virus challenge exposure; however, from calves that shed BVDV in groups B, C, and D, at least one calf in each group was not viremic at any time point after virus challenge exposure (Table 6). Bovine herpesvirus-1 was not detected in serum samples but viral DNA was isolated from nasal swab samples of experimental calves during the first 14 days of virus challenge exposure. Positive BoHV-1 results on nasal swabs were detected on days 7, 9, and 14 in control calves, days 9 and 6 in group B, day 9 and 14 in group C, days 3 and 7 in group D, and day 7 in group E. The number of calves shedding BoHV-1 after virus challenge exposure was not significantly different among treatment groups (A: n=3; B: n=3; C: n=1; D: n=2; E n=1, $P > 0.05$).

*3.4 Sequences of BVDV-positive WBC samples*

Alignment of BVDV consensus sequences obtained from RT-nPCR positive WBC samples revealed >95% identity with the 5' UTR of the sequences obtained from the 6 PI cattle. Type 1a BVDV was identified in 2 calves from the control group and 3 calves from group D, type 1b BVDV was identified in 5 calves from the control group, and type 2 BVDV was identified in 2 calves each from the control, C, and D groups, respectively, and 1 calf from group B.

| Group | Number of viremic calves (%) | Number calves that shed virus (%) |
|---|---|---|
| **A** | 9/11 (81.8)a | 5 /11(45.5) |
| **B** | 1/11 (9.1)b | 2/11 (18.1) |
| **C** | 2/10 (20)b | 1/10 (10) |
| **D** | 4/11 (45.4)ab | 3 /11 (27.2) |
| **E** | 0b | 0 |

Table 6. Total number and proportion (%) of calves with viremia and BVDV shedding in nasal secretions during virus exposure challenge. The number and proportion of BVDV viremic (BVDV positive on WBC or serum samples) calves and calves that shed BVDV (BVDV RT-nPCR positive nasal swab samples) during virus challenge exposure was higher in control and D groups compared with groups B, C, and E. Groups with different letters are significantly different ($P < 0.05$)

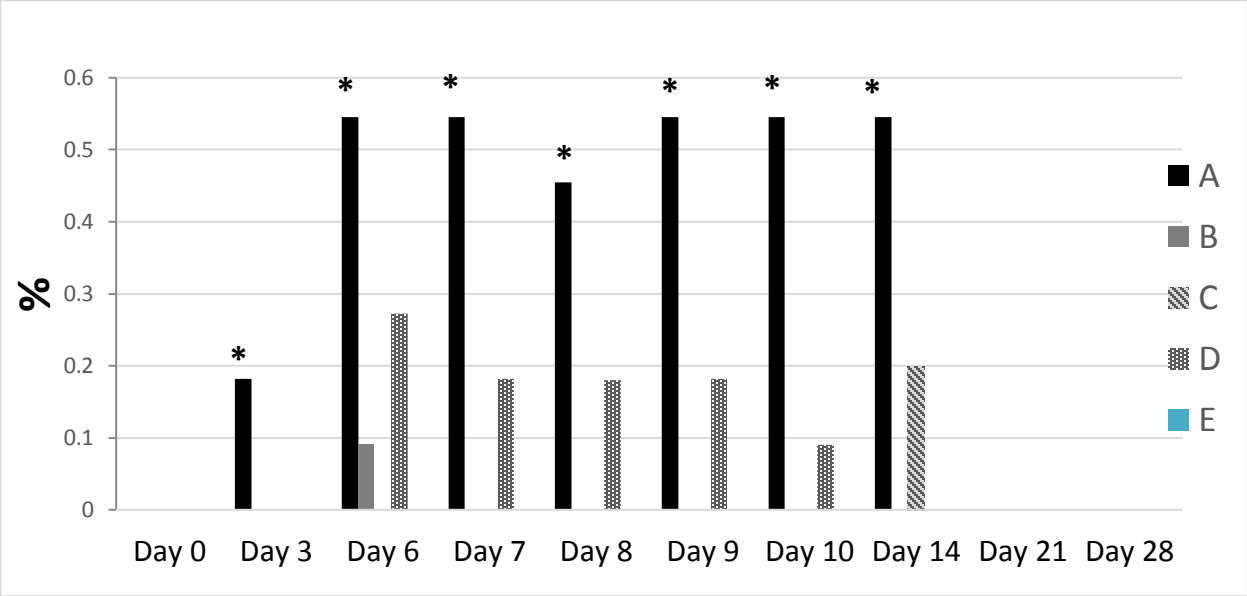


Figure 20. Proportion of calves positive to BVDV in WBC or serum samples per time period. The proportion of vaccinated (B, C, D, and E) and control (A) calves with a positive BVDV result in WBC or serum samples was significantly higher in the control (A) group on days 6, 7, 8, 9, 10, and 14 after challenge compared with groups B, C, and E. On days 6, 7, 8, and 9 after challenge the proportion of calves with positive BVDV WBC and/or serum samples was not significantly different between group D and controls. (*$P < 0.05$).

*3.5 Virus neutralization*

Thirty days prior to vaccination (day -75) and at a median calf age of 61.5 days, the mean titers of maternally-derived BVDV 1, BVDV 2, and BoHV-1 antibodies were similar between groups (Table 7). At vaccination (day -45), decay of maternally-derived antibodies for BVDV 1, BVDV 2 and BoHV-1 was detected in all groups. At day 0 (first day of virus challenge exposure), calves from groups B, C, D, and E had higher GM of serum BVDV 1a and BVDV 1b antibody titers compared with the control group; mean

antibody titers to BVDV 2 was higher in calves from group E compared to controls; however, groups B, C, D, and control had similar mean antibody titers to BVDV 2. At day 28 (last day of virus challenge exposure), calves from groups B, C, and E had higher GM antibody titers to BVDV 1a compared to control calves. Significant differences were not found in the GM antibody titers to BVDV 1a between groups D and control. Geometric mean BVDV 1b antibody titers were similar in all treatment groups. However, group E had higher mean BVDV 2 antibody titers compared to the control group. There was a significant decay of BoHV-1 antibody titers over time (day 0 to day 28) in all groups; however, differences among vaccinated (B, C, D, and E) and control (A) groups were not statistically significant (Table 7).

Among vaccinated groups, seroconversion as defined as a 4-fold or greater rise in antibody titers to BVDV 1a, BVDV 1b, and BVDV 2 after vaccination (day 0) was observed in varied proportions of calves from groups B, C, D, and E (Table 8). None of the calves in group A (control) seroconverted to BVDV 1a, BVDV 1b, or BVDV 2 at day 0. A higher proportion of calves in groups B, C, and E seroconverted to BVDV 1a and BVDV 1b when compared with group D (Table 8). Similarly, a higher proportion of calves from groups B and E seroconverted to BVDV 2 compared to groups C and D. Seroconversion to BoHV-1 was not observed in any of the groups at any time of the study.

| | | Day of Study | | | |
|---|---|---|---|---|---|
| **Virus** | **Group** | **-75** | **- 45** | **0** | **28** |
| **BVDV 1a** | **A** | 77[a] (14.9 – 398.9) | 19.3[b] (7.3 – 95.7) | 13.1[c] (3.3 – 51.6) | 46.5[e] (19 – 113.7) |
| | **B** | 93.05[a] (24.7 – 349) | 32[b] (9 – 112.9) | 238.8[d] (147 – 390.7) | 657.1[f] (284 – 1520) |
| | **C** | 128[a] (28.6 – 568) | 39.4[b] (12.2 – 126.2) | 337.8[d] (179 – 630.3) | 724.0[f] (252.4 - 2062) |
| | **D** | 67.6[a] (9.9 – 464.6) | 32.0[b] (6.2 – 162) | 56.0[d] (26.7 – 118.6) | 186.0[e] (68.1 – 508.4) |
| | **E** | 77[a] (15.5 – 382.6) | 34.05[b] (7.7 – 149) | 186.1[d] (109 – 315.2) | 792.35[f] (436 – |
| | | | | | |
| **BVDV 1b** | **A** | _ | 10.9[a] (3.7 – 31.7) | 6.2[b] (2.2 – 16.9) | 68.1[d] (17.2 – 268.7) |
| | **B** | _ | 17[a] (3.5 – 81) | 128[c] (88.6 – 183.5) | 328.5[d] (122.8 – 879.2) |
| | **C** | _ | 14.9[a] (5.1 – 42.8) | 84.4[c] (41.3 – 171.2) | 445.7[d] (129.7 – 1520) |
| | **D** | _ | 14.92[a] (4.8 – 55.3) | 21.85[c] (11.5 – 41.3) | 93[d] (30 – 288) |
| | **E** | _ | 13.2[a] (3.6 – 47.8) | 77.2[c] (42.5 – 139) | 256[d] (98.3 – 661.7) |
| | | | | | |
| **BVDV 2** | **A** | 145[a] (25.1 – 837.5) | 35[b] (9 – 128) | 24.7[c] (7.2 – 85.6) | 68[e] (29.2 – 158.6) |
| | **B** | 99[a] (25.6 – 382.6) | 18[b] (8.5 – 38) | 56[c] (24 – 132.5) | 328.5[e] (153.2 – 699.4) |
| | **C** | 111.4[a] (29.6 – | 32[b] (10.6 – 95.6) | 73.5[c] (38 – 141) | 256[e] (62.2 – 1045) |
| | **D** | 52.7[a] (8.57 – 324) | 17[b] (4.4 – 64.9) | 82[c] (31.3 – 213.7) | 290[e] (73 – 1152) |
| | **E** | 87.4[a] (178.3 – | 34[b] (8.9 – 129.8) | 1585[d] (765 – 3281) | 2797[f] (1428.2 – 5480) |
| | | | | | |
| **BoHV-1** | **A** | 13.2[a] (3.9 – 44.6) | 6.6[b] (1.9 – 22.3) | 2.7[c] (1.2 – 6) | 1.86[d] (1 – 3.3) |
| | **B** | 17[a] (5.5 – 51.6) | 7.4[b] (2.8 – 20) | 4[c] (2 – 7.9) | 2.26[d] (1 – 4.9) |
| | **C** | 18.4[a] (6.3 – 53) | 10.5[b] (3.8 – 29.2) | 2.3[c] (1.2 – 4.2) | 2.29[d] (1.2 – 3.8) |
| | **D** | 8.5[a] (2.37 – 30.2) | 5.8[b] (1.75 – 19.4) | 2.9[c] (1.2 – 6.9) | 2.26[d] (1.2 – 4.2) |
| | **E** | 10.2[a] (3.2 – 32.9) | 5[b] (1.8 – 11.4) | 4.5[c] (1 – 7.8) | 2.56[d] (1 – 6.8) |

Table 7. Geometric mean (95% CI) of virus neutralizing serum antibody titers to BVDV 1a, BVDV 1b, BVDV 2, and BoHV-1 from vaccinated (B, C, D and D) and unvaccinated (A) calves at each time period. Significant effect of time (day) and treatment on antibody

levels is indicated by superscript lower case letters. Time periods (day of study) not connected by the same letter are significantly different ($P < 0.05$). Groups not connected with the control group (A) by the same letter (superscript) at each time period (day of study) are significantly different compared to the control group ($P < 0.05$). _ Not determined

| Group | BVDV 1a | BVDV 1b | BVDV 2 | BoHV-1 |
|---|---|---|---|---|
| **A** | 0/11(0) | 0/11(0) | 0/11(0) | 0/11(0) |
| **B** | 8/11 (72.7)[a] | 8/11 (72.7)[a] | 8/11 (72.7)[a] | 0/11[a] |
| **C** | 7/10 (70)[a] | 6/10 (60)[a] | 3/10 (30)[b] | 0/11[a] |
| **D** | 3/11 (27.2)[b] | 3/11 (27.2)[b] | 3/11 (27.2)[b] | 0/11[a] |
| **E** | 5/11 (45.4)[a] | 7/11 (63.6)[a] | 9/11 (81.8)[a] | 0/11[a] |

Table 8. Number and proportion (%) of calves that demonstrated a 4 fold or greater rise in individual antibody levels to BVDV 1a, BVDV 1b, BVDV 2, and BoHV-1 following vaccination. Groups not connected by the same letter within each virus are significantly different ($P < 0.05$).

*3.6 Hematology*

The mean WBC count decreased significantly over time (from day 0 to day 14) in all groups ($P < 0.0001$); Significant differences in WBC were only observed at day 0 when calves from the control group had a significantly higher WBC count compared to calves from group E ($P = 0.0233$). At the same time, the mean WBC from calves in

groups B, C, and D were not significantly different compared to control calves ($P > 0.05$, Figure 21). The mean platelet counts were not significantly different between treatment groups (Figure 22).

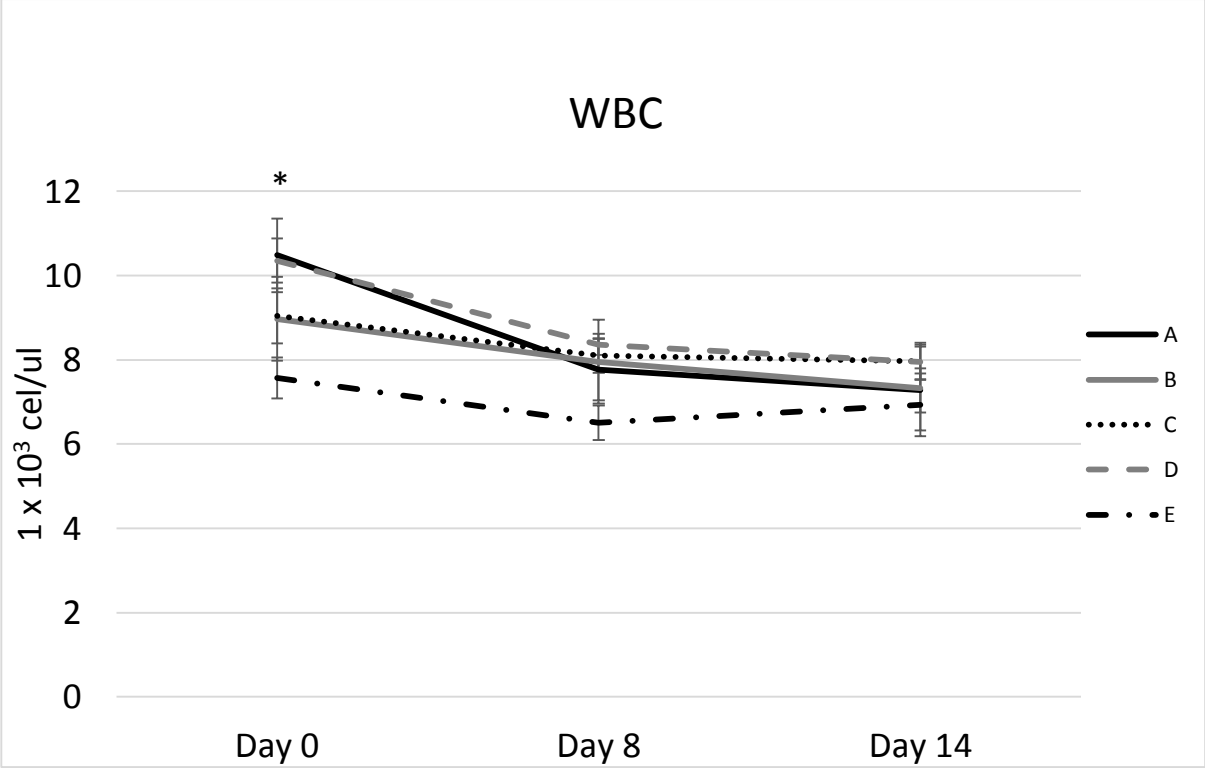


Figure 21. Mean WBC +/- SEM among study calves following challenge exposure to BVDV and BoHV-1. The mean WBC was significantly higher at day 0 in calves from the control group compared with calves from group E (*$P < 0.023$); however, significant differences among groups were not found at any other time point. There was a significant effect of time on the mean WBC ($P < 0.00001$) from day 0 to day 14 as all groups had significantly less WBC at day 14 compared with day 0.

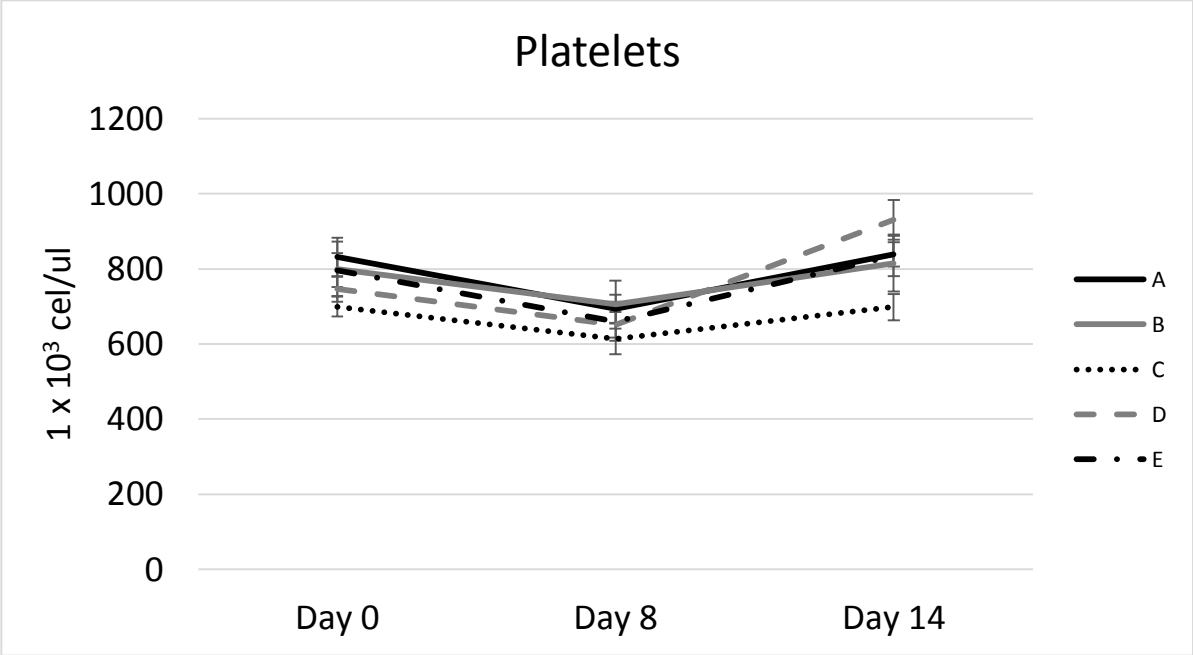


Figure 22. Mean platelet count +/- SEM among study calves following challenge exposure to BVDV and BoHV-1. The mean platelet counts among study calves were not significantly different between groups ($P > 0.05$).

## Discussion

The stress of weaning, shipping, and exposure to respiratory viruses through commingling are important risk factors for BRDC in beef calves (Griffin 1997). In the present study, early weaned beef calves were vaccinated at weaning and then shipped and simultaneously exposed to cattle PI with BVDV and calves acutely infected with BoHV-1 in an attempt to simulate the environmental and management conditions frequently associated with the natural presentation of BRDC. In the present study, clinical signs of severe respiratory disease or mortality were not observed in any of the calves.

Additionally, clinical scores, rectal temperatures, body weights, WBC, and platelet counts were not significantly different among vaccinated and control calves. This suggests that maternally-derived immunity to BVDV and BoHV-1 provided protection from clinical disease associated with acute BVDV and BoHV-1 infections. Transmission of BVDV was evident under the virus exposure model used in this study as several of the experimental calves developed viremia and BVDV shedding. Additionally, the nucleotide sequences (5′ untranslated region of BVDV) amplified from viremic calves had a high homology (>95%) with the nucleotide sequences observed in BVDV strains from the 6 PI cattle. Transmission of BoHV-1 under this virus exposure model was less than optimal as BoHV-1was not isolated from serum samples and virus DNA was recovered from nasal swab samples of few unvaccinated control calves. It is possible that the quantity of virus shed by the calves intravenously inoculated with BoHV-1 did not allow an effective transmission of the virus to a larger number of experimental calves. We speculate that the peak of nasal shedding of BoHV-1 in these inoculated calves might have occurred prior to commingling with the experimental animals decreasing the risk for virus transmission and infection. Peak of shedding of BoHV-1 after experimental inoculation of naïve calves occurs typically 5-6 days post infection (Peters et al. 2004; Ellis et al. 2005; Ellis et al. 2009). Therefore, in this study the peak of BoHV-1 shedding might have already passed by initiation of commingling. Neutralization of BoHV-1 by maternally-derived BoHV-1 antibodies present in calves under study may offer another explanation for the absence of viremia and reduced number of calves shedding BoHV-1 (Ellis et al. 1996; Peters et al. 2004).

Despite the absence of severe clinical signs of disease, there was a marked difference in the proportion of calves that became viremic and shed BVDV among treatment groups; however, multiple primary outcomes included in the present study could have influenced results observed in viremia and virus shedding in experimental calves as multiplicity could have increased type 1 error during data analysis. Unvaccinated control and group D calves became viremic and shed BVDV in greater proportions than calves from groups B, C, and E. Interestingly, not all vaccinated calves (B, C, D, and E groups) that shed BVDV were viremic after virus challenge exposure and some of them did not shed BVDV until day 21 or 28 of challenge suggesting the possibility of contamination of nasal secretions rather than a true BVDV infection. This could have been the consequence of continuous exposure to BVDV shed by PI cattle during the 28-day virus challenge exposure model; however, we believe that the higher levels of BVDV 1a, BVDV 1b, and BVDV 2 antibody titers observed in calves vaccinated with B, C, and E played a critical role in reducing viremia and BVDV shedding after commingling with PI cattle. Several studies have indicated that calves with low serum BVDV antibody titers (GM < 64) at experimental challenge with BVDV demonstrate higher risk of clinical disease, viremia, and viral shedding compared to calves with higher BVDV serum antibody titers (Howard et al. 1989; Cortese et al. 1998c; Ellis et al. 2001).

The lower GM antibody titers and higher number of calves in group D that were viremic and shedding BVDV compared to groups B, C, and E could be associated, we hypothesize, with the presence of highly specific maternal antibodies induced by previous vaccination of dams with vaccine D. These specific vaccine D maternally-derived

antibodies could have limited the induction of BVDV-neutralizing antibodies and negatively affected protection from viremia and BVDV shedding in group D calves; however, this hypothesis needs to be confirmed with further scientific data.

Factors such as age and level of maternally-derived immunity at the time of vaccination influence the humoral responses of young calves vaccinated with MLV respiratory vaccines (Menanteau-Horta et al. 1985; Ellis et al. 2001; Peters et al. 2004; Fulton et al. 2004). The presence of a minimal titer of maternally-derived BoHV-1 antibodies interferes with antibody production and seroconversion in vaccinated calves (Brar et al. 1978; Moerman et al. 1994). This could explain the continuous decay and absence of BoHV-1 seroconversion observed in experimental calves in the present study. A higher proportion of calves at a median age at vaccination of 93.5 days seroconverted to BVDV 1a and BVDV 1b in groups B, C, and E, and a higher proportion of calves in groups B and E seroconverted to BVDV 2. The antibody responses to BVDV 1a and BVDV 1b were consistently higher in calves vaccinated with B, C, and E compared to the control group. Interestingly, the proportion of calves with viremia and BVDV shedding was lower in the same groups (B, C, and E). This suggests that antibody responses induced by vaccines B, C, and E could have reduced viremia and BVDV shedding after virus challenge.

Protection against clinical disease, viremia, and virus shedding of young calves previously vaccinated with MLV BVDV vaccines in the presence of maternally-derived immunity and subsequently challenged with virulent BVDV has been associated with priming of specific T lymphocyte populations that develop cell-mediated memory responses once maternal antibodies have decayed (Ridpath et al. 2003; Endsley et al.

2004; Zimmerman et al. 2006); However in a recent study, vaccination of very young calves (3-days-old) with a MLV vaccine containing BVDV and subsequently challenged with virulent virus 7-9 months after vaccination protected calves against clinical disease but failed to protect against viremia and viral shedding as 80% of vaccinated calves became viremic (Stevens et al. 2011). This suggests that priming of T cell responses by early vaccination with MLV vaccines does not always protect against viremia after BVDV challenge in young calves. In the present study, high antibody titers to BVDV 1a, BVDV 1b, and BVDV 2 were associated with reduced viremia and virus shedding after BVDV exposure. This indicates that priming of B cell responses and antibody production may be as important as priming T cell mediated immunity after vaccination of young calves with MLV respiratory vaccines in providing overall protection against BVDV challenge.

Vaccination of early weaned beef calves in the presence of maternally-derived immunity resulted in increased antibody responses and reduction of viremia and BVDV shedding. Reduction of viremia and BVDV shedding after vaccination of young calves with MLV vaccines has been associated with reduction of BVDV transmission and extent of BVDV exposure (Thurmond et al. 2001; Peters et al. 2004). The ability of MLV vaccines to reduce transmission of BVDV could have a great economic impact on vaccination programs of early weaned beef calves marketed to operations such as stocker/backgrounding units and feedlots as it could be associated with reduction of BVDV transmission and decreased incidence of BRDC after arrival.

# Summary and Conclusions

Bovine viral diarrhea virus (BVDV) among other respiratory viruses plays a critical role in the presentation of bovine respiratory disease complex (BRDC) in young calves. Vaccination of calves against BVDV is considered a strategic management measure of herd health programs of beef and dairy operations to reduce economic losses due to BRDC. The presence of maternally-derived antibodies against BVDV at the time of vaccination interferes with the induction of adequate antibody responses in vaccinated calves. High BVDV antibody levels from colostrum or induced by vaccination have been demonstrated to reduce the incidence of BRDC in beef operations (Fulton et al. 2002). Additionally, high antibody responses to vaccination in young calves have been associated with decreased viremia and nasal shedding following challenge with BVDV (Howard et al. 1992; Peters et al. 2004). Decrease viremia and virus shedding after exposure to BVDV is a desirable outcome of vaccination as it could result in a reduction of BRDC in young calves. The objective of the research presented in this dissertation was to determine the persistency of maternally-derived immunity against common respiratory viruses such as BVDV, BoHV-1, BRSV, and PI3V and to evaluate the effect of different levels of maternally-derived immunity in the induction of humoral responses to vaccination with MLV vaccines containing BVDV and BoHV-1. Additionally, we wanted to determine the ability of different multivalent MLV vaccines containing BVDV

and BoHV-1 to protect against clinical disease, reduce viremia, and prevent virus shedding in calves exposed to BVDV or BVDV and BoHV-1 forty-five days after vaccination.

The objective of the initial experiment was to determine the persistency and duration of maternally-derived antibodies against respiratory viruses including BVDV 1, BVDV 2, BoHV-1, BRSV, and PI3V in calves that received maternal colostrum or a colostrum replacement product at birth. Results from this experiment indicated that calves that receive maternal colostrum present a high variability on the initial antibody titers and duration of immunity against respiratory viruses. In contrast, the levels of antibodies against the same viruses transmitted by the colostrum replacement product were more uniform as it was the duration of immunity especially with respect to BVDV 1 and BVDV 2. These results suggest that calves that received maternal colostrum at birth might become susceptible to acute infection with respiratory viruses earlier in life compared with calves that receive the colostrum replacement product. Therefore calves that receive maternal colostrum at birth could benefit from early vaccination. In contrast, calves that receive the colostrum replacer product might have a prolonged duration of maternally-derived immunity against respiratory viruses and vaccination in these calves could be delayed.

During the second study, the ability of three different MLV vaccines containing BVDV to induce antibody responses and protect against clinical disease, viremia, and virus shedding was evaluated in early weaned beef calves challenged with virulent BVDV 2 45 days after vaccination. Calves vaccinated with vaccines B and C demonstrated higher antibody responses to vaccination and decreased proportion of

individuals with viremia and BVDV shedding compared with unvaccinated control calves. Differences in the ability of vaccines to protect against viremia and BVDV shedding was detected during this study as calves with higher antibody responses to vaccination (B and C groups) demonstrated lower levels of viremia and BVDV shedding. The results of this study suggest that the ability of MLV BVDV vaccines to prime B cell responses and induce antibody production could result in reduced numbers of viremic calves and prevent BVDV shedding. Reduction of viremia and BVDV shedding could decrease BVDV transmission and therefore might reduce incidence of BRDC in young calves.

A third focus of this research was to evaluate the ability of four different MLV vaccines containing BVDV and BoHV-1 to protect against clinical disease, reduce viremia, and prevent virus shedding in early weaned beef calves simultaneously exposed to 6 cattle PI with BVDV and 8 calves acutely infected with BoHV-1 45 days following vaccination. In this study, calves vaccinated with vaccines B, C, and E demonstrated superior antibody responses to vaccination and presented lower proportion of individuals with viremia and BVDV shedding after challenge. Differences in antibody responses to BoHV-1 were not detected among groups and BoHV-1 was not isolated from any of the experimental calves. The results of this study indicate that vaccination of young calves in the presence of maternally-derived immunity against BVDV could result in priming of B cells and antibody production. Calves that respond with higher levels of BVDV antibodies after vaccination have lower levels of viremia and BVDV shedding reducing the risk of virus transmission and disease.

In summary, the high variation on the duration of BVDV specific antibody levels absorbed from maternal colostrum could affect the humoral responses to vaccination in young calves; however, prevention of seroconversion after vaccination due to interference exerted by maternal antibodies do not prevent priming of B cell responses that increase antibody production. Based on results from the research presented in this dissertation we suggest that the ability of different MLV vaccines containing BVDV to induce activation of B cell responses and antibody production could result in reduction of viremia and BVDV shedding in young calves exposed to BVDV. Reduction of viremia and BVDV shedding after vaccination and subsequent challenge-exposure with BVDV is a highly desired outcome of vaccination IFOMA as it could decrease the risk of BVDV transmission and infection among calves from the herd. Reduction of acute BVDV infection prevents immunosuppression and could reduce the overall incidence of BRDC.

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