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# Host response to bovine viral diarrhea virus and interactions with infectious agents in the feedlot and breeding herd

Robert W. Fulton\*

Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, 250 McElroy Hall, Oklahoma State University, Stillwater, OK 74078, USA

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## ABSTRACT

Bovine viral diarrhea viruses (BVDV) have significant impact on beef and dairy production worldwide. The infections are widespread in the cattle populations, and in many production systems, vaccinations are utilized. BVDV strains have the hallmark of adversely affecting the immune system's many components, both the innate and acquired systems. While BVDV do cause primary infections and disease, their role in the pathogenesis of other agents underscores the complexity of viral–bacterial synergy. A greater understanding of the role of the persistently infected (PI) animal resulting from susceptible females infected at a critical stage of pregnancy has permitted acknowledgment of a major source of infection to susceptible animals. Not only do we understand the role of the PI in transmitting infections and complicating other infections, but we now focus attempts to better diagnose and remove the PI animal. Vaccinations now address the need to have an immune population, especially the breeding females in the herd. Biosecurity, detection and removal of the PI, and effective vaccinations are tools for potential successful BVDV control.

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## 1. Introduction

Bovine viral diarrhea virus (BVDV) infections occur in cattle in various forms. The BVDV infections can be manifested by single organ involvement, involve several organ systems, and/or work in concert with other infectious agents to cause clinical illness [1]. BVDV have tropism for many organs, including respiratory tract, digestive tract, lymphoid system, reproductive tract, and the fetus. Thus, it is overly simplistic to classify infections into specific diseases for BVDV, except for persistently infected (PI) calves born to susceptible females exposed during pregnancy, and mucosal disease (MD) in PI calves superinfected with a second and related cytopathic strain of BVDV.

The role of BVDV in synergistic or mixed infections with other agents is most likely due to its well known immunosuppression. In a review by Pogeiter [2] and study of BVDV [3] on innate immunity, there are numerous references to the effects on the lymphoid organs and reductions in B-cells, T-cells, and neutrophils, impaired bacteria killing and decreased chemotaxis, decreased lymphocyte proliferation, decrease immunoglobulin secretion into the circulation. Likewise there may be reduction in T-helper and T-cytotoxic

lymphocytes. In addition to immunosuppression in the bovine acquired immune system (humoral and cell mediated), the innate immune system of the bovine respiratory tract can be impaired by BVDV [4]. Thus, BVDV is often associated with secondary or co-infections with other agents.

## 2. BVDV subtypes and their infections in the United States

BVDV are a diverse group of viruses, both by antigenic and genomic properties [1,5,6]. There are two major genotypes, BVDV1 and BVDV2. There is a further subclassification with subgenotypes based on genomic and antigenic differences: twelve BVDV1 (a-1) and 2 BVDV2 (a and b) [5]. In the U.S. there are three major subtypes, BVDV-1a, 1b, and 2a. There is a single report of a BVDV-2b in the U.S. [7]. The BVDV are also categorized based on the presence or absence of viral induced cytopathology in infected cell cultures, the cytopathic (cp) or noncytopathic (ncp) biotypes. The ncp strains predominant in natural infections with the recovery from diagnostic laboratory accessions indicating approximately 90% ncp compared to approximately 10% cp strains [8]. While the ncp strains predominant in the natural infections in cattle, interestingly almost all of the U.S. licensed vaccines contain cp strains [1].

The distribution of BVDV subtypes in the U.S. cattle is largely based on the genomic testing for the subgenotypes of isolates from the diagnostic laboratory accessions representing ill cattle or cattle

\* Tel./fax: +1 405 744 8170.

E-mail address: [robert.fulton@okstate.edu](mailto:robert.fulton@okstate.edu).

**Table 1**  
Disease forms with bovine viral diarrhoea viruses – immunosuppression associated with many forms.

Acute-transient infections (inapparent)
Respiratory – Acute uncomplicated and co-infections with other agents
Digestive tract – Acute disease and co-infections
Thrombocytopenia/hemorrhagic form
Mucosal disease
Reproductive tract/fetal infections
Persistently infected (PI) cattle

presented for necropsy testing, or the detection of PI cattle with subsequent genotyping of the PI strain. Antibody testing will not always give a true indication of the infections as BVDV vaccines are widely used, and the vaccines induce antibodies indistinguishable from naturally infections with the serologic tests in place. Antibody surveys, even in the absence of vaccinations, may indicate how widespread the PI cattle are in the respective population which exposes susceptible cattle which respond with active infections detected by seropositive status.

Testing for PI cattle in various populations, both by geography and/or management (beef breeding herds, stockers, or feedlots; dairy milking herds or calf raisers) has been reported. Calves from beef herds in five states were tested for BVDV PI status with 2.7% of the herds with at least one PI calf and 0.17% of the calves tested were PI [9]. In that study, 7% of the PI calves' dams were also PI. Another study tested for PI cattle in selected herds and 1.7% of the cattle were PI with BVDV and 6 of 66 (9.1%) herds had PI cattle [10]. In a state-wide study of calves in Iowa beef breeding herds, the PI rate for BVDV was 0.09% with 3.9% of the herds with a PI animal [11]. Calves from beef breeding herds in south central Oklahoma and north Texas were tested for BVDV PI status with 0.55% (25 of 4530) of the calves PI and 5 of 30 (16.7%) of the herds with one or more PI calves [12]. In that study, 5% of the PI calves had a PI dam. This was the initial study in the U.S. to report the BVDV subtypes in the PI calves from a beef breeding herd, with all isolates as BVDV-1b. A study of feedlot cattle indicated 0.3% of the cattle entering a feedlot were BVDV PI [13]. Subsequently a study of cattle from southeastern states in the U.S. entering a feedlot evaluated various tests for PI cattle and detected 88 of 21,743 (0.4%) were PI [14]. This study was the initial study in the U.S. to report the distribution of BVDV subtypes in PI cattle entering a feedlot: BVDV-1b, 77.9%; BVDV-1a, 11.6%; and BVDV-2a, 10.5%. In an Iowa study cattle entering a feedlot, 10 of 5041 (0.2%) of the cattle were PI [15]. There are limited studies on the prevalence of BVDV in the dairy industry. A study of young bull dairy calves in Arizona reported that 15 of 3010 (0.49%) of the calves were PI, and in a companion study of beef calves only 1/1096 (0.09%) were PI [16]. Thus, it appears that these various studies with cattle under different managements (calves in breeding herds, feedlots, beef operations and dairy calves) range of positive PI calves was 0.09%–0.55%.

The distribution of the BVDV subgenotypes in the U.S. reported above was supported by other published studies [8,17–19]. A study reported in 2002 indicated the distribution of BVDV from cases in the northwestern U.S. beef and dairy samples with 18.5% BVDV-1a, 40.7% BVDV-1b, and 40.7% BVDV2 (the subtype was not listed) [17]. Of isolates positive for BVDV from an Oklahoma diagnostic laboratory accessions representing clinical ill cattle and necropsy cases, primarily from Oklahoma, other states represented included Kansas, Texas, and Arkansas. From these positives, BVDV-1b, (45.8%); BVDV-1a, (28.2%); and BVDV-2a, (26.0%) were identified [8]. There were no affinities for a subtype for any particular organ system from those identified as the source of the same: respiratory, digestive, mixed/multiple organs, abortions, or PI. All BVDV subtypes were isolated from PI animals. From diagnostic laboratory

accessions from dairy operations of 16 states, principally from the eastern states in the U.S., and originating from bulk milk samples and infected cattle, there were 49.1% BVDV-1b; 11.3% BVDV-1a; and 39.33% BVDV-2a [18]. Thus from two different diagnostic laboratories there were similarities in the distribution of the three BVDV subtypes. A later study of Australian and U.S. isolates reported on the distribution of BVDV subtypes from PI cattle in southwest U.S. feedlots and similarly to the report of reference [14], among the 514 isolates there were 12.1% BVDV-1a, 75.3% BVDV-1b, and 12.6% BVDV-2a [19]. Thus it appears in the U.S., that the predominant BVDV subtype in the U.S. is BVDV-1b, based on analysis of diagnostic laboratory accessions and studies from PI cattle. The predominance of BVDV-1b, over BVDV-1a and BVDV-2a, is of considerable interest as the USDA licensed MLV and killed vaccines available and marketed in the U.S. contain, in most all cases, BVDV-1a and BVDV-2a [20].

### 3. BVDV disease forms

BVDV infections occur in cattle in various manifestations or disease forms (Table 1) [1]. Creating unique disease forms is nearly impossible as the virus may infect multiple organ systems. Coupled with the ability of BVDV to cause immunosuppression in the transiently infected animal often resulting in mixed infections with other agents makes classification more difficult. A hallmark of BVDV infections is the immunosuppression of the host response to infections resulting in increased incidence and virulence of secondary infections with other viruses, bacteria, and mycoplasmas. And BVDV itself may not infect a single organ system, often infecting multiple organs resulting in systemic disease.

#### 3.1. Acute-transient infections (inapparent)

As with many viruses, BVDV infections in the postnatal susceptible calf are most often inapparent. One important feature is in the pregnant female and BVDV infections during pregnancy, and discussed below, is the fact that an infection in the pregnant female may lead to detrimental effects in the fetus without the dam exhibiting clinical signs. Numerous surveys of nonvaccinated cattle in unvaccinated herds indicate antibody positive cattle. The younger calf is usually the target of the acute infections after the loss of maternal antibodies following ingestion of colostrum. The mean half-life of passively acquired BVDV antibodies is approximately 23 days [21]. The actual age the calf becomes susceptible is dependent on the amount of BVDV antibodies absorbed from the colostrums and the dose of virus. The incubation in the susceptible, virus exposed calf is 5–7 days with viremia typically less than 15 days, but may be longer dependent on virus strain, stress, and other pathogens [22].

#### 3.2. Respiratory disease

BVDV may cause respiratory infection in acute primary (uncomplicated with other agents) with disease and clinical signs postexposure as shown by experimental infections [23,24]. In general, this viral–bacterial synergy occurs as viruses predispose the bovine to secondary infections by compromising the host defense mechanism by multiple mechanisms: (1) the upper respiratory tract with the nasal turbinate mucosal epithelial cells damaged with altered mucociliary clearance with bacterial attachment, growth, and colonization; (2) the tracheal mucosal epithelial cells are damaged with the mucociliary apparatus compromised with the bacteria attachment, growth, and colonization; (3) the innate defense system of the lung compromised, with viral infections of the macrophages and neutrophils (major

phagocytic cells of the innate host defense system) either depleted, destroyed, or with impaired antibacterial properties; and (4) the acquired immune system such as T-cell (cell mediated) or B-cell (humoral) systems suppressed (1). BVDV strains have the potential to alter any one or multiple mechanisms resulting in impaired host responses to other infections. BVDV has been shown by experimental challenge with both BVDV exposure and bacterial challenge to work in concert with other agents including bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine rotavirus and *Mannheimia haemolytica* [23,25–29]. Additionally, there have been studies in feedlot cattle with naturally occurring BRD indicating infections occur with BVDV along with other pathogens including parainfluenza-3 virus (PI-3V), BRSV, bovine adenovirus (BAV), *M. haemolytica*, *Pasteurella multocida*, and *Mycoplasma* spp. [30–32].

Finally, for supporting evidence of BVDV associated with BRD, there are reports of fatal feedlot pneumonias with the recovery of BVDV with other agents including BHV-1, PI-3V, bovine coronavirus (BCV), *M. haemolytica*, *P. multocida*, *Histophilus somni*, and various *Mycoplasma* spp. [7,33–35]. In a study of feedlot pneumonias in an Oklahoma feedlot, the three U.S. BVDV subtypes recovered from the lungs were BVDV-1a, BVDV-1b, and BVDV-2a [7]. Interestingly, the vaccine virus, cp BVDV-1a NADL, was recovered in lungs of calves dying soon after processing in the feedlot. Viruses recovered shortly after MLV vaccination, should be differentiated as to vaccine or field strain origin. There were relationships determined among the infectious agents, lesions, and feedlot performance in that study [7], with significance statistically ( $p$  value  $\leq 0.05$ ) in several comparisons. Calves infected with BVDV field strains became ill sooner and died earlier in the feedlot than those BVDV negative. There were subtype differences as well, BVDV-1b ncp cattle were treated earlier, had shorter treatment interval (day of initial treatment until death [fatal disease onset]) and died earlier in the feedlot (day of death in the feedlot). BVDV-1a ncp cattle were treated earlier as well. Cattle with ncp BVDV-1b isolated from the lungs were related to the recovery of *M. haemolytica*. A similar finding was observed for the recovery of BVDV-1a cp vaccine strain and *M. haemolytica*. BVDV-1a cp vaccine strain was positive in calves with acute pneumonia. The recovery of BVDV-1a cp strain from acute cases and in those treated early in the feedlot may be a temporal event associated with MLV vaccination rather than a virulence factor with lesion development. Vaccine viruses were sometimes recovered within days of vaccination [1].

### 3.3. Digestive tract

Digestive tract disease caused by BVDV can occur in most any age from neonate to adult [1]. This primary form or transient infection usually occurs in cattle six to 24 months of age [35]. The acute form is manifested by fever, anorexia, depression, with possibly ulcers/erosions in the oral mucosa and on the tongue, and potentially diarrhea [35]. Due to the immunosuppressive effects of the virus, it is not unusual to find concurrent infections with *E.coli*, *Salmonella* spp. cryptosporidiosis, rotavirus, or BCV. The virus damages the epithelial structures of the digestive tract including ulcers and erosions, and potentially, but not always, in the entire digestive tract. Because of the affinity for lymphoid tissues, the Peyer's patches in the intestine may be affected. An acute BVDV disease with extensive mucosal lesions (larynx, esophagus, glossitis), bronchopneumonia, high morbidity, and mortality was observed in a commercial feedlot [36]. The affected cattle were negative for PI status as determined by ear notch negative by ELISA. The affected cattle were positive for BVDV-2a and BVDV-1b. The cattle likely were exposed to PI cattle in transit to BVDV-2a or to a PI with BVDV-1b in the same shipment. The gross

and microscopic lesions of acute BVDV in this case were not distinguishable by observed lesions from mucosal disease. The acute digestive tract diseases are most likely caused by ncp strains of BVDV. This in contrast to mucosal disease described below.

### 3.4. Thrombocytopenia/hemorrhagic form

Calves may undergo an acute infection with a hemorrhagic syndrome [35,37,38]. The disease is characterized by thrombocytopenia, bloody diarrhea, hemorrhages on visible mucous membranes, bleeding from injection sites, and death [37,38]. ncp BVDV strains are the biotype involved with this disease form. The mechanism for the hemorrhage and thrombocytopenia is not fully understood. The disease is often fatal.

### 3.5. Mucosal disease

Mucosal disease (MD) was thought by many to be the "classical" form of BVDV with low morbidity and high mortality. MD is the result of a persistently infected calf (discussed below under fetal infections), infected with an ncp strain, developing disease after infection with a related cp virus. Infection with the related cp strain is most likely the result of the original ncp mutating/evolving to the cp strain [35]. The disease is characterized by severe digestive tract disease with ulcers/erosions potentially throughout the entire digestive tract, skin lesions, and hoof lesions including the interdigital space. The disease is uniformly fatal. The disease could be similar to a severe form of acute digestive tract disease, but is differentiated from the acute disease by two features: (1) in MD, both the cp and ncp are isolated from the affected animal or from necropsy tissues; and (2) the animal with MD will have evidence of PI status, i.e., positive skin test samples detected by Immunohistochemistry (IHC) or antigen capture ELISA (ACE).

There was concern that MLV BVDV vaccination may have contributed to MD as the MLV BVDV vaccines in almost all cases contain the cp biotype [1]. The theory was that the cp vaccine strain would have been related to the ncp strain from the PI animal. However with such a low rate of PI cattle entering the feedlot (less than 1%), and most all cattle receiving an MLV BVDV vaccine at entry processing, there is an extremely low incidence of MD in the feedlot to support the connection to the MLV BVDV vaccine. Also, one study demonstrated that vaccination of PI calves with different MLV vaccines did not induce MD associated with MLV vaccines [39]. In reality the calves responded with an active antibody response to the BVDV immunogens in the vaccines.

### 3.6. Reproductive tract/fetal infections

The outcome of infection in the susceptible heifer/cow exposed to BVDV is dependent upon the gestational age of the pregnancy [1,35,40]. Exposure from day -9 prior to early pregnancy, suggests BVDV in the susceptible female may have negative effects on conception and/or implantation of the fertilized ovum resulting lowered conception rates and the repeat cycling observed clinically. Abortions may occur resulting from fetal exposure between stage of day 45–175 [35] with both ncp and cp infections [40]. Most label indications in the U.S. for BVDV MLV vaccines do not list approval for use in pregnant cows/heifers [20]. There are selected MLV vaccines in the U.S. with label indications for use in pregnant females, but the approval process and indication have stipulations for that specific vaccine to have been given prior to breeding and within a specified time, usually within a year [20].

Congenital defects and malformations may occur as a result of BVDV infection between days 100–150 and are dependent on the critical age for organ development [40]. A wide range of defects

may occur including cerebellar hypoplasia, microencephaly, hydrocephalus, hydranencephaly, porencephaly, hypomyelination, cataracts, microphthalmia, retinal degeneration, optic neuritis, thymic hypoplasia, hypotrichosis, deranged osteogenesis, brachygnathism, and growth retardation [references in citation 40]. Clinicians should be aware of a variety of potential congenital defects should BVDV circulate among pregnant females as not all of the pregnant females will be of the same gestational age of pregnancy.

### 3.7. Persistently infected calves

Fetuses infected with an NCP strain between day 42–125 may survive and be carried to term, born alive, and survive as a lifetime shedder of virus [41]. These calves are referred to as persistently infected (PI). Not all susceptible females exposed at this interval will give birth to a PI calf; however, some may be aborted, or develop congenital defects [40]. The cp biotype fetal infections do not result in PI calves, as they do not appear to cause persistent infections in the fetus and/or establish immunotolerance to the infecting biotype. The PI calf is the most important reservoir of the virus, shedding virus in all the secretions/excretions during the lifetime of the animal [1,42]. The PI calves are immunotolerant to the infecting ncp strain and may respond with antibody production to either heterologous field strains or vaccine strains [39,42].

Fetal infections in the last trimester may occur after organogenesis is complete and after development of the immune system [1]. These infections in the last trimester may result in calves born with BVDV antibodies in the precolostral serum and without detectable virus as the fetal immune system clears the virus [40]. The term “congenitally infected” has been used for the calves that are virus negative, BVDV antibody positive (at birth). They appear to be at greater risk for illness postnatal in life than calves born antibody negative [43]. The extent/prevalence of these congenitally infected calves remains to be determined.

Recently a report of a disease outbreak in a dairy herd underscores the point that a variety of disease outcomes may result with BVDV fetal infections [44]. BVDV-1b was isolated from Holstein dairy calves from a dairy herd experiencing premature births, brachygnathism, growth retardation, malformations of the brain and cranium, are rare extracranial skeletal malformations.

## 4. Immunity to BVDV in acute infections and response to vaccinations

Cattle may respond to BVDV infections with humoral (antibodies) and cell mediated (T-cell) responses after exposure to experimental viral challenge or exposure to naturally occurring field strains and vaccinations. Calves exposed to intranasal administered virus representing several nonvaccinal BVDV strains in individual calves responded with serum antibody production [45]. Calves seronegative to BVDV exposed intranasally to either NCP or CP strains responded with an active response with increased T-cells post infection [46]. Interestingly calves with BVDV antibodies derived maternally responded with activation T-cell responses after exposure to virulent BVDV [47]. Systemic active immune responses as indicated above reveal humoral and cell mediated immunity after exposure to BVDV when serums and circulating leukocytes are evaluated. A study was designed to evaluate the active immune response by the lung in exposed cattle [48]. Calves received BVDV intrabronchially and sequential bronchoalveolar lavage samples postexposure were evaluated for humoral and cell mediated responses. The exposed calves responded with increased anti-BVDV IgA and anti-BVDV IgG in the lavage fluids. Total numbers of CD4<sup>+</sup> and CD8<sup>+</sup> in the lavage fluids also

increased. The increase in BVDV antibodies in the lung and the increase in T-cells coincided with the clearance of the challenge BVDV. A secondary viral challenge resulted in increase in BVDV antibodies and increased T-cells in the lung.

Cattle respond to MLV and killed BVDV vaccines with both antibody and T-cell responses. Susceptible calves receiving an MLV vaccine containing BVDV-1a and BVDV-2a responded with increased serum BVDV antibodies and increased BVDV activated T-cell responses [49]. Also it was observed that calves with maternal antibodies to BVDV responded with activated T-cells after MLV BVDV vaccination [50]. Historically, it was thought that killed (inactivated) viral vaccines would not stimulate T-cell responses, often giving the MLV an advantage over the killed vaccines. Recently a study was reported whereby calves were given a BVDV killed vaccine containing BVDV-1a and BVDV-2a immunogens and the immune response was investigated [51]. The calves responded with increased serum antibodies to BVDV-1a and BVDV-2a and also antigen specific T-cell subsets.

Studies of naturally occurring infections in feedlot cattle have indicated that BVDV infections often result when mixed source cattle are commingled. Cattle, not receiving BVDV vaccines, with acute samples collected at entry at day 0 followed by convalescent samples 4–6 weeks later, were shown to have seroconverted to BVDV [30,31]. In some cases there was no evidence of a PI animal in the group (30), suggesting exposure either immediately prior to commingling and shipment or infections due to acute infections. The ability of PI cattle to expose large numbers of susceptible cattle was shown in multiple studies under feedlot conditions [31,52,53]. The PI BVDV cattle are often very efficient in infecting susceptible contacts as 70%–100% susceptible contacts became infected after exposure in the pens with PI cattle [52,53].

Immunity to BVDV is measured by the ability to resist challenge. The mechanisms of recovery or the protective host defense mechanisms for BVDV infections most likely include both the BVDV specific antibodies and activated T-cell subsets along with the innate immunity [3,54,55]. Traditionally, passive immunity had been considered protective when cattle are challenged with virulent viruses. However a study indicated that a protective immune response was mounted in calves with passive immunity to BVDV, but the protection was not reflected by serum BVDV antibodies [55]. Calves with colostral antibodies were infected with virulent virus and were protected, yet the calves did not increase the serum antibody levels and they eventually declined. The calves were still protected when infected with virulent virus while having undetectable antibodies at time of exposure. The ability of calves to mount an active T-cell subset response to MLV vaccine while possessing maternal antibodies suggests that activated BVDV T-cells help confer protection to BVDV [47,55].

Colostrum derived immunity is a factor in host protection against viral challenge and potentially inhibits a response to vaccination. The efficacy of colostral immunity is dependent on the level of antibodies present when the animal is challenged or vaccinated. It is difficult to ascribe a specific titer for protection or susceptibility as there are different tests used by laboratories and the challenge virus may vary along with animal phenotype, management or genetic variation. Colostral antibodies that are absorbed by the calf have a half-life in the serum of approximately 23 days [1,21]. In a study evaluating the effect of maternal immunity on vaccination with a killed vaccine containing BVDV-1a and BVDV-2a, all calves with a passive serum titer of 128 or higher to both BVDV-1a and BVDV-2a failed to develop an active antibody response to either immunogen after two doses [21]. At a titer of 64 only half of the calves developed increased antibody titer to either immunogen. Protection is afforded by the presence of passively derived serum antibodies when the calves are challenged with

a highly virulent BVDV strain. Calves with neutralizing antibody titer of 256 or lower had fever and systemic spread of virus post-challenge, and calves with titer below 16 had severe clinical disease [56]. When calves with neutralizing titers to BVDV-1b and below were exposed to PI calves with BVDV-1b, some, but not all became viremic [52]. A study using BVDV-1b PI calves as the challenge model indicated that pregnant vaccinated heifers (BVDV-1a and BVDV-2a immunogens) with serum titers of 128 to BVDV-1b and above were protected against fetal infections [57]. Eight control heifers and two vaccinated heifers with titers <4 had infected fetuses, and the other two control heifers control heifers aborted before the collection date. Antibody titers to BVDV-1b of 8–64 gave equivocal results. Most fetuses from heifers with titers of 8–64 were protected, yet four fetuses became infected (0/1 fetus of a heifer with titer of 8 became infected, 1/5 fetuses of heifers with a titer of 16 became infected, 1/9 fetuses of heifers with a titer of 32 became infected, and 2/9 fetuses of heifers with a titer of 64 became infected). One heifer with a BVDV-1b titer of 32 did have a normal fetus, but did become infected (viremic) after exposure to PI BVDV-1b calves. In a study to evaluate animal health status for cattle at feedlot entry to predict feedlot performance and carcass evaluation, antibody levels to several BRD pathogens were measured [58]. On either an individual animal or herd basis for the calves, there were predictors for performance (such as measurement of immunity to pathogens). Calves with low levels of BVDV-1a and BVDV-2a antibodies had increased treatment costs and decreased net value returned to the owner. Calves treated twice or more had lower levels of BVDV-1a than those treated once or not at all.

### 5. Role of PI animal in co-infections with other agents

The PI animal is believed to be the most important source of infection for the postnatal animal. The PI animals are lifetime shedders with high levels of virus in the nasal secretions and other excretions [1,14,42]. The source of the virus excreted via nasal secretion by PI cattle is illustrated by the examination of the respiratory tract (nasal mucosa to lung) of PI calves with BVDV-2a [59]. Using IHC, BVDV antigen was detected in the respiratory tract in squamous and columnar epithelium, mixed tubuloalveolar glands of the nasal mucosa, secretory cells and ductular epithelium, nasal mucus secreting cells, and tracheobronchial glands, as well as alveolar macrophages. Thus the secretions of the nasal cavity of the PI animal, no doubt contain considerable virus. High titers of BVDV are found in the nasal swab materials taken from PI calves [14,42]. In an eleven month long study of PI cattle, cattle with BVDV-1a, 1b, and 2a, the level of infectivity in the nasal swabs was titrated and ranged from log<sub>10</sub> 2.90 to 7.85 per ml of nasal fluids.

The question arises what might be the role of cattle acutely infected with BVDV in transmitting virus. Challenge studies focus on detection of virus (systemic infection) in the blood such as peripheral leukocytes. Little or no information is available as to the quantity of virus in a nasal swab from an acutely infected animal, as the reports are solely qualitative (positive or negative). In order to look at the dynamics of infection, nonvaccinated animals may be exposed to vaccinated animals to look for shedding of vaccine virus to the controls as detected by seroconversion or perhaps viremia [60]. Field studies using cattle in feedlots with viral isolations and serology gave useful information on acute infections. In two studies calves were purchased from an auction and sent to a nearby order buyer barn and shipped to feedlots. The calves were from commingled, mixed sources, and did not receive BVDV vaccinations and were held in the feedlots for 32 and 33 days respectively with blood and nasal swabs collected weekly for virus isolation [31]. In this study there were ranch calves mixed with the order buyer calves at

the feedlot and included one BVDV-1b PI calf. To illustrate the short range of infection for acutely infected animals, the days from which BVDV was isolated from acutely infected animals was found to be days 4–11 in the blood, and days 10–12 from the nasal swabs, thus a short range of infectivity. The PI calf was positive in all blood and nasal swabs throughout the study. In a later study with order buyer, auction market calves and no BVDV PI calves in the shipment, the range of isolations from the acutely infected calves were day 3–25 in the blood, and day 18 for the only calf positive in the nasal swab. Another study using PI BVDV challenged calves, some previously vaccinated and nonvaccinated controls, indicated acute infections with virus isolation positives: blood positive, days 7–21, and nasal swab, days 3–7 [52]. A study using PI BVDV calves in a challenge of vaccinates and nonvaccinates, the range of infectivity for acutely infected calves was: blood positive, days 6–13 and day 6–13 for the nasal samples [53]. Thus the range of naturally occurring infection in acutely infected calves is short based on these studies: day 3–13 in the blood (rarely one at day 25) and day 6–13 in nasal swabs. Potential acutely infected animals may be shedding in external secretions, but the amount of virus being shed is likely below the threshold for infectivity of susceptible contacts.

The presence of PI cattle in the feedlot has been shown to have an adverse effect on production. A feedlot study reported 0.3% of the cattle entering the feedlot were PI [13]. Using penmates of PI cattle and those in adjacent pens, the risk of initial treatment for BRD was 43% for those cattle exposed to PI cattle, 2.6% of the chronically ill cattle were PI, 2.5% of the fatal cases in the feedlot were PI, and 15.9% of initial cases of BRD were attributed to PI exposure [13]. As noted prior, PI cattle are extremely efficient in transmitting virus to susceptible contacts. In two studies, the PI cattle were able to infect 70%–100% of the susceptible contacts in the same pen continuously with the PI cattle [52,53]. A study was designed to evaluate various levels of exposure to PI animals in a starter feedlot (66 days) and the effects on feedlot performance [61]. The protocols to minimize exposure to PI cattle in a feedlot used the procedure of identifying the PI calf and then either leaving the PI calf in the pen, removing the PI from the pen, and following those pens along with the pens adjacent to the pen with the PI or the pen with the PI removed. This study involved 21,743 cattle, with 86 PI animals (0.4%). There were 74 of 172 (43%) of the pens having at least 1 PI animal. If the PI cattle were not removed, 107 of 172 (62.2%) of the pens would have had direct exposure to a PI in the pen or a PI in the adjacent pen. Performance outcomes improved slightly as the risk of exposure decreased. Comparing the effects of PI exposure were the direct exposure (PI calf remaining in the pen and pen adjacent to a pen with a PI remaining) and pens without exposure to PI cattle, there was a total cost per animal on the basis of population exposure to PI BVDV cattle of \$93.52 (\$5.26 for fatalities and \$88.26 in performance losses). This study gave insight in the potential for decreasing the adverse effects of PI calf exposure in a starter feedlot by reducing exposure after identification and removal of the PI calf from the lot/pen.

This study represented a very large population of cattle with several BVDV genotypes present in PI cattle (86 of 21,743 PI). These had a distribution of 77.9% BVDV-1b, 11.6% BVDV-1a, and 10.5% BVDV-2a. The large general population and the large number of pens involved illustrates the variability of the individual pen's performance (cost of gain [COG]), suggesting the potential of the virulence of the BVDV strain in the pen or exposing the adjacent pen. Variability was greater in lots with direct exposure to PI animal. Twenty of fifty lots (40%) of the direct exposure group had values equal to or less than the mean of the unexposed group. However only 1 of 64 (1.6%) lots of the unexposed group had higher COG than the mean of the direct exposed group. This point

underscores the issue that in studies there should be large numbers of pens and PI cattle to account for the variability, which would include virulence of the virus as well as animal-to-animal variation.

The fate of PI cattle is illustrated in the above study under feedlot conditions [61]. There were 22 PI cattle that died in the study and were examined by necropsy. These animals had a 25.6% fatality rate compared to 2.4% of the non-PI cattle. Of the PI animals, there were 14 of 22 (63.6%) attributable to MD, 6 of 22 (27.3%) to BRD, 1 (4.5%) to bloat, and 1 that was not identified. Additionally in this study, 4 of 37 (10.8%) PI cattle were considered chronically ill and sold as salvage slaughter compared to 3.6% of the non-PI cattle. The mean length of illness for fatal MD cases was 23 days and for those dying of BRD, 38 days. Many PI cattle in feedlot may die early, yet it is known that some may complete the finishing period and are marketed. Likewise, veterinarians and producers for beef and dairy cowherds and milking operations have reported that some PI cattle may live for years.

PI cattle themselves are immunotolerant to the ncp strain with which they were infected in utero. However, PI calves may still respond to heterologous BVDV strains (natural infections or vaccinations) with increased neutralizing antibodies [39,42], yet they may not respond to other immunogens such as *M. haemolytica* vaccine [39]. With the survivability/death losses of the PI cattle illustrated in the feedlot studies above (61) and the infections associated with their death, it is not unexpected to say they are immunocompromised. In a year long feedlot study of fatal pneumonias, calves dying of BRD were necropsied and samples examined for histopathologic lesions, infectious agents recovered and feedlot performance evaluated [7]. In that study there were 5.3% of the BRD fatal cases that were PI. PI cattle were associated, ( $P = \text{value } 0.05$ ) with the recovery of *Archanobacterium pyogenes*, recovery of BVDV field strains, lesions of lobar bronchopneumonia, and cases of chronic pneumonia [7].

The commingling of the PI animal under production settings has been used in recent investigations to determine how the presence of PI animals impact production and to determine vaccine efficacy. These studies: (1) demonstrate how PI calves may predispose cattle to other infections; and (2) demonstrating the ability to serve as challenge to susceptible cattle in studies evaluating vaccine efficacy against infections in susceptible calves and against fetal infection/disease [26,31,32,62]. A study was conducted to demonstrate that the viral–bacterial synergy could be enhanced by the use of PI BVDV cattle prior to bacterial challenge [26]. Susceptible calves were exposed to PI BVDV-1b calves for 72 h and then challenged intratracheally with *M. haemolytica* [26]. The PI BVDV-1b challenge resulted in reduced antibody response to *M. haemolytica* leukotoxin postchallenge and increased rectal temperature. This system with BVDV PI calf exposure prior to *M. haemolytica* challenges offers a useful model for the pathogenesis of viral–bacteria synergy in BRD. Two studies using either BVDV-1b or BVDV-2a PI calves to challenge vaccinates and nonvaccinate controls provided evidence that vaccination protected against systemic infection (viremia) subsequent to PI calf exposure [31,32]. Additional studies were done using PI calves to challenge vaccinates and nonvaccinates as measured by protection against fetal infection/disease [57,62,63]. In those studies the PI calf challenge resulted in both the infection of the controls as measured by viremia and infected fetuses or calves born to the control nonvaccinates. Thus, the use of PI calves in vaccine efficacy studies is mirrors the naturally occurring infections in cattle operations.

## 6. Treatment of PI animals

Treatment of the PI animal has little potential for achieving complete virus free status. The PI animal by definition is

immunotolerant and has virus present in all body systems and most tissues and secretes/excretes large quantities of virus. Current antiviral compounds are not effective in decreasing viral titers. An example of an attempt to treat PI calves illustrated the lack of success [64]. PI calves were treated with a recombinant human interferon  $\alpha$ -2a every other day for 12 weeks. There was no antiviral activity in the PI calves resulting from the interferon treatment, and the calves developed adverse immunologic and hematologic effects indicating toxic effects of the treatment. Not only will selection of potential antivirals be a challenge, but federal regulators involved with approval will not likely approve such treatments due to safety issues including food safety.

## 7. Prevention of adverse consequences of BVDV infections

Prevention of BVDV requires three components: (1) identification of the source of infection in cattle, particularly the PI animal and the removal of those animals; (2) biosecurity: effective implementation and continued maintenance of barriers to entry of the virus; and (3) an immune population based on proper vaccination. Critical issues related to the control of BVDV along with the knowledge gaps relevant to BVDV are outlined in a recent review article [5]. The identification of the PI animal and its removal is the beginning of the control program. There are numerous diagnostic tests available to detect PI animals, including use of viral isolation in cell culture using peripheral blood, identification of BVDV antigen in formalin fixed skin tissues by immunohistochemistry (HC), identification of BVDV antigen in supernatants from skin tissue samples (antigen capture ELISA [ACE]), and PCR tests (gel based and real time) using supernatants of skin tissue samples [65]. A limited number of diagnostic laboratories provide PCR testing of bulk milk samples from individual dairies to identify herd infections. For many, the viral isolation in cell cultures on two consecutive tests 3–4 weeks apart is the gold standard for PI status. The two positive tests 3–4 weeks apart are to rule out acute infections. Others have used IHC of formalin fixed tissues as the standard as well for PI status. Pooling of samples for PCR testing has been used by some laboratories to reduce the cost of testing, whereas other labs have not chosen to offer the pooled testing due to validation and technical issues. If a pooled test is negative, then the entire number of represented animals is considered negative; but if a positive pool is found, then the individual samples must be tested. Many veterinarians and producers prefer to use tests that are designed for one animal at a time, such as ACE on skin samples or IHC of fixed tissue. Animals found to be PI are recommended to be removed from the population and ideally isolated to prevent exposure to other cattle, especially susceptible females. The PI cattle are permitted to be processed for meat consumption unless treated with antibiotics or other drugs that requires a holding period before processing. It is highly recommended that PI cattle not reenter the market system for return to other herds. The final option is euthanasia.

Biosecurity against BVDV entry/reentry is similar for other diseases such as Johne's disease. Ideally, cattle from the outside (bulls, heifers/cows, steers) should test negative for BVDV as a condition of purchase. However, if cattle are not tested prior to entry to the premises they should be isolated from resident animals, particularly pregnant animals, until the test results are known. Cattle returning from livestock shows or bull or heifer raisers should be isolated as well for approximately 3–4 weeks. A special comment applies to entering pregnant heifers/cows. They may test negative, but could be carrying an infected fetus. Thus, pregnant females entering the herd should be held in isolation until the calf is born and tested for BVDV. Also, cattle breeding operations using purchased semen for artificial insemination should obtain the semen from facilities known for BVDV free procedures and animal

testing. The use of embryo transfer for advancement of bovine genetics requires vigilance to use BVDV free donors and recipients as well as BVDV free serum. Obtaining recipient females requires the same testing as if the animal was purchased for breeding. Should the operation maintain a breeding herd and purchase stocker calves for forage, those purchased animals should be kept separate from the breeding herd or if mixing has taken place, they should be tested for BVDV.

Most emphasis for biosecurity has focused on the cattle and the PI animal to prevent exposure to BVDV. In recent years evidence shows that deer may be infected with BVDV via exposure to PI cattle [66]. Also susceptible pregnant deer exposed to a PI deer gave birth to live fawns and one fawn was found to be PI [67]. Thus the interface of wildlife and domestic cattle raises the issue of inter-species transmission of BVDV under natural or range conditions for the beef or dairy herd. The role of the PI deer or acutely infected deer in the transmission of BVDV to susceptible cattle deserves investigation under current production systems, both beef and dairy, to determine the relative role of infected deer that might impact any control program.

Vaccination is a critical point in BVDV control. An immune cattle population is important for control programs, but also to lessen impact of disease, improve production for the beef and dairy producer, and enhance economic return to the producers. Vaccines should be effective for protection against acute infections/diseases, but equally if not more important, protection against fetal infection with resulting PI calves. To control BVDV effectively, the cycle of PI calves needs to be broken as the PI animals are considered by most to be the major reservoir of infection. There are both killed and MLV vaccines available in the U.S. [1]. BVDV MLV vaccines are capable of inducing a wide range of antibodies to several strains [68,69]. Also BVDV MLV may have an advantage over killed vaccines where one dose gives rapid protection as early as 3 days after vaccination [70]. The antibody response to both MLV and killed BVDV vaccines will decline over time, but the animal with low antibody levels will have an anamnestic response following revaccination [71]. Current vaccination protocols call for annual revaccination. Currently for licensure, the vaccine must pass efficacy challenge studies using acute challenge in susceptible cattle. However, more recently in the U.S., selected vaccines have received U.S.D.A approval for label claims for protection against fetal infections and/or disease [1]. MLV vaccines have gained more use in recent times over killed vaccines. Vaccination of calves with two doses is usually recommended followed by another dose prior to breeding. The vaccination of pregnant cows with MLV should be only used based on the approval/licensure for the respective vaccine. BVDV MLV vaccination in the susceptible cow could result in fetal infection and disease for the fetus [1,20,35,40]. Vaccinations against BVDV are critical to control, but must be used appropriately to affect control. Biosecurity and the elimination of the PI animal are good partners with vaccinations in the BVDV control programs.

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