# “A Tale of BVD”


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## Introduction

The evolving cattle industry has peaked at an all time high in facing more diseases than ever, with bovine viral diarrhea (BVD) being one of the most prevalent infectious diseases. Increasing efforts in attention to bovine viral diarrhea) virus (BVDV) in the last several years allowed comprehension of the major economical and financial losses producers experience due to the clinical manifestations of bovine viral diarrhea virus.

Bovine viral diarrhea, a Pestivirus of the family Flavivirdae, refers to a group of single stranded RNA viruses that is further divided into two genotypes (BVDV-1 and BVDV-2) (Larson, R). These genotypes are further subdivided as cytopathogenic and non-cytopathogenic based on their activity in cultured epithelial cells, with non-cytopathogenic BVDV predominating in nature. (Larson, Robert L.) Strains of BVDV have been isolated from ruminants in all continents with the exception of Antarctica. (Ridpath, Julia F.)

Possibility of acute infection resulting in seroconversion of healthy, immunocompetent, cattle is reported (Lanyon, Sasha R.,). However, BVD is primarily transmitted and maintained in herds by persistently infected (PI) individuals through production and shedding large amounts of infective virus (Lanyon, Sasha R.,). Infection of the dam during early gestation results in fetal infection, and subsequently the possibility of a persistently infected calf. Economic losses also occur through immune suppression via viral shedding from PI individuals (Lanyon, Sasha R.) .

Majority of control is aimed toward elimination of persistently infected cattle and thus the source of continuing infection. Diagnostic testing is readily available to confirm clinical cases, establish disease prevalence, and identify apparently normal, but persistently infected animals in a herd. Efforts toward affordable screening, vaccination, implementing good

biosecurity and management practices may provide adequate protection against the detrimental effects from bovine viral diarrhea (Lanyon, Sasha R.).

**History and Presentation**

Bovine Viral diarrhea is ubiquitous in North America and is extremely variable in presentation. (White , Brad) First reports were observed in New York dairies in 1946, by Dr. Francis Fox of Cornell University (Ridpath, J.f.). The disease was described as “rinderpest-like” disease displaying leukopenia, diarrhea, high fever, dehydration, gastrointestional erosions, and hemorrhages in tissues (Ridpath, J.f.). A case report observed five herds with a 33-88% morbidity and 4-8% mortality.. In addition, fetal abortions were noted from 10 days to 3 months following infection (Lanyon, Sasha R.).

In the 1950’s another disease given the name mucosal disease was discovered. Clinical presentation was similar to this “rinderpest like disease” however gastrointestional lesions were more severe and it could not be transmitted experimentally unlike the 1946 case report. This condition also detailed low morbidity and high mortality rates, thus, not initially thought to have the same causative agents (Ridpath, J.f.). It was named mucosal disease. Late 1950’s led to isolation of the noncytopathic virus as the causative agent of BVD. Years later, a cytopathic strain was discovered from a mucosal disease case. Thus, cross neutralization studies led to the discovery that BVD and Mucosal disease were different disease manifestations of infection of the same agent.

Observation of BVDV in fetal bovine serum paved the understanding of in utero infections. In the 1980’s USDA’s National Animal Disease Center reported persistent infection and immune tolerance in apparently healthy adult animals. Calves born to PI cows mounted a

persistent infection at birth, thus, confirming maternal transmission. Additionally, exposure of a seronegative cow to noncytopathic BVDV between 42 and 125 days gestation also lead to the development of a PI calf (Ridpath, J.f.). BVDV's etiology unraveled through molecular basis for biotypes and clinical presentations of its disease processes.

Bovine viral diarrhea virus may present as arguably the most complicated combination of clinical presentations. (Ridpath, J.f.) A detailed way to keep presentations straight is to divide BVD infections into 3 categories including: acute infections, infections in pregnancy, persistent infections, and mucosal disease (White, Brad).

*Acute BVD infection*

Acute BVDV infections most commonly persist as subclinical to mild symptoms. Often these individuals are not obviously ill. However, since BVDV is immunosuppressive, clinical signs seen may be attributed to other agents. Often clinical signs are seen between six months to two years of age. Clinical signs may include: high morbidity, low mortality, biphasic fever, depression, decreased milk production, transient decrease in appetence. Possible other signs may include: increased respiratory rate with excessive nasal secretion, lacrimation, and possible bouts of diarrhea (White , Brad). Short-term leukopenia, lymphopenia and/or thrombocytopenia, apoptosis in the thymus, and immunosuppression can also occur. (Lanyon, Sasha R.,).

*Infections in pregnancy*

Reproductive effects of acute BVDV include reduction in conception rates, early embryonic loss, abortions, and congenital defects (Lanyon, Sasha R.,). Different signs depend on the stage of fetal development (White,Brad). Infection at fertilization results in early embryonic loss. Within the first four months of gestation, BVDV infection may cause resorption, abortion, growth retardation, or

persistent infection in fetus (White, Brad). If the fetus is infected during four to six months of development and ultimately makes it to full term, congenital malformations of the eye and central nervous systems may occur. These signs could include bilateral cataracts, brachygnathism, hydrocephalous, and cerebellar hypoplasia resulting in ataxic calves at birth (Lanyon, Sasha R.). Acute infections of sexually active bulls result in reduction of sperm motility and abnormal morphology (Paton). Research has documented that BVDV can remain in semen for up to 2.75 years after initial infection. However, results conclude that no transmission was discovered from infected bull (Lanyon, Sasha R.). A persistently infected bull can clinically displayed signs of testicular hypoplasia (Borel).

*Persistent Infections*

Persistent infections from in utero exposure during day 42-125 of gestation is the most clinically important due to the shedding of large amounts of BVDV. Maintaining a vicious cycle of virus exposure unfortunately capable of presenting as a clinically healthy individual (White , Brad). Some may exhibit stunted growth, weakness, ill-thrift, and become prone to enteric and respiratory problems (Lanyon, Sasha R). In feedlot situations, BVDV viremia or seroconversion has been associated with respiratory disease outbreaks (Larson, Robert L.). Persistently infected calves with normal vitals have been shown to have thyroid hormone concentrations be significantly lower than healthy calves (Larsson et al).

*Mucosal Disease*

Occasionally an animal that is persistently infected with noncytopathic BVDv may become superinfected with cytopathic BVDv and develop severe, highly fatal form known as mucosal disease (White , Brad ). Animals with acute mucosal disease may present with a fever, severe diarrhea, leukopenia, and erosions/ulcerations of the mouth and nose (White , Brad). Secondary

bacterial infections and diarrhea may also persist (Lanyon, Sasha R). Ultimately the outcome is death, in which such event may occur within a few days, or be protracted and take a few weeks (Bolin).

**Pathophysiology**

With the vast array of clinical presentations BVDV infections display, understanding the pathogenesis of the disease in order to select an appropriate diagnostic approach is essential. Bovine Viral Diarrhea virus is divided into non-cytopathogenic and cytopathogenic biotypes based on effects on cultured cells rather than in the infected host. Cytopathic strains induce cell death in cultured cells, while non-cytopahtogenic types do not. (Lanyon, Sasha R) These biotypes also differ in production of an extra nonstructural protein known as NS3 (Ridpath, J.f.). They make up acute infections, fetal infections, persistent infections, and mucosal disease process's involved in bovine viral diarrhea (Lanyon, Sasha R).

In acute infections, non-pregnant non-immune cattle, entail the non-cytopathogenic biotype BVDV resulting in transient viremia. CD46 has been shown to be the receptor on macrophage and lymphocyte host cell membranes where BVDV gains entry (Maurer). This is usually initiated on day 3 post-infection until immunity develops, roughly 2 weeks later. The most common spread of infection between cattle includes nose-to-nose or sexual contact with persistently infected individuals. Flies, aerosolized virus, and contaminated equipment/pens have also been documented as a means of transmission. Acute infection of a naïve animal results in a transient viremia for 10-14 days and likely immunosuppression. In turn, immunosuppression is associated with direct effects of BVDV on circulating T and B lymphocytes and apoptosis of lymphocytes in gut associated lymphoid tissue (Pedrera). In decreasing order, localization of virus in enterocytes, peyer's patches, thymus, spleen, and lymph nodes are associated in nasal

infection of healthy calves with noncytopathic BVDv. Clearance of virus may take 9-13 days post-infection by T cell cell death of lymphocytes. Diarrhea observed in some acutely infected cattle is presumptively due to infection of myenteric and submucosal ganglia of gastrointestinal tract and interruption of normal intestinal neural function. Full recovery of acute infections usually takes three weeks to gain clearance. However, outliners may appear in infected, recovered, and now immunocompetent individuals where these continue to carry virus in peripheral blood mononuclear cells for 98 days or more (Lanyon, Sasha R).

Fetal infections pathogenesis depends on the age of fetus when the BVDV first occurred. Infection of the embryo does not occur during the vesicle stage of pregnancy due to the fact that the virus cannot penetrate the zona pellucida (Lanyon, Sasha R). Infection may begin as early as 29-41 days of gestation. These signs of infection may result in early embryonic death thus, poor reproductive performance rates in cattle (Lanyon, Sasha R). Persistently infected calves can be established in utero 42-125 days of gestation (Ridpath, J.f.). Teratogenic effects of the fetus may be seen 80-150 days of gestation. These signs are characterized by cerebellar atrophy, ocular degeneration, brachygnathism, pseudocyst formation in the brain, thymus, bone, and lung retardation. Abortion may be seen during this time with no concurrent effects passed to the cow (Lanyon, Sasha R).

Persistently infected cattle are unique in the fact that rather invading the adaptive immune response of an immune-competent animal, noncytopathogenic biotype is able to establish a persistent infection by invading the fetus early in its intrauterine development (Larson, Robert L.). This is established before development of a competent immune response, therefore, establishing immunotolerance that is specific for the persisting viral strain (Larson, Robert L.). The window for in utero infection development of a PI calf is varied from individual to individual, however; is

reported as early as 25 days to as late as 125 days. Establishment of PI individuals is from the noncytopathic biotype inhibiting the induction of type 1 interferon in the fetus. This allows the virus to invade and survive in the host. These calves are not able to mount an antibody response to clear the virus and lasts the entire life of the animal (Lanyon, Sasha R.,). The PI calf then exposes others by producing and shedding huge amounts of the very infective virus through all excretions and secretions. This includes: milk, semen, saliva, nasal discharges, urine, blood, and aerosols. Persistently infected animals may contain wide distribution of BDVV in lymph nodes, gastrointestinal tract epithelia and lymphoid cells, lungs, skin, thymus, and brain. Also, studies detail BVDV has been found to be localized in the oocytes of PI females, a possible reason of the logic behind calves born to PI cows are always persistently infected themselves. Distribution to the central nervous system is characteristic as well (Lanyon, Sasha R). Persistent infections cause the most economic losses due to the very high and persistent viremia associated with its process (Larson, Robert L.). Horizontal transmission of BVDV to seronegative cattle have been shown to occur only after one hour of direct contact with a single animal (Larson, Robert L.).

Lastly, mucosal disease manifestations always lead to mortality in affected cattle (White, Brad). Mucosal disease only develops from persistently infected cattle and is established when a noncytopathic biotype becomes infected with cytopathic biotype (White , Brad). It may also be naturally transmitted between individual animals that are persistently infected with the same homologous groups noncytopoathic strains isolate (Lanyon, Sasha R.,). All cytopathic biotypes produce protein NS3, whereas with noncytopathic biotypes only the uncleaved form, NS2/3 can be detected (Peterhans).

**Diagnostic Approach**

Accurately identifying BVDV in herds is a vital key in stopping and eradicating the infection from a specific herd or region (Lanyon, Sasha R.,). BVDV diagnostics have focused on the detection of PI animals. Various diagnostic methods for identifying BVDV herd infections include: serology, virus isolation from serum or other tissue, reverse transcription polymerase chain reaction amplification (RT-PCR), antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA), and immunohistochemistry (IHC) staining of viral antigen in skin biopsy (White , Brad).

Virus isolation has historically and currently noted as the gold standard for diagnosis of BVDV in herds. The use of PCR is becoming increasingly common, with Real time- PCR (RT-PCR) currently being widely accepted as the standard for BVDV diagnosis (Lanyon, Sasha R). Newer research is reporting that RT-PCR has many advantages over virus isolation in the fact that it is less time consuming and less expensive for producers. RT-PCR is highly sensitive and a variety of samples may be taken (blood, milk, follicular fluid, saliva, and tissue samples) with prolong storage time acceptable. Application of primers specific to the $5^0$ untranslated region has shown that it is possible to successfully identify type I and type II BVDV using RT-PCR (Letellier). RT-PCR can detect persistent infections however, repeat testing at 4 weeks intervals is advised with successive positive results indicating a persist infection (Lanyon, Sasha R.). RT-PCR has been practically used on pooled serum samples to indicate persistently infective cattle as well as acute infection in a herd. A positive pooled serum sample RT-PCR indicates that BVDV infection is occurring. However, a negative result doesn't rule out a BVDV infection. The same principle can be applied to bulk tank milk sample of a milking herd (Lanyon, Sasha R.). Due to the nature of the disease and test, RT-PCR sensitivity and specificity of 100% is approachable (Larson, Robert L.).

The Antigen- enzyme linked immunosorbent assay (Ag-ELISA) test is ideal for herd screening due to its simplicity. Since last reviewed in 2004 (Saliki and Dubovi), multiple BVDV Ag-ELISAs currently being commercially made (Lanyon, Sasha R). These diagnostic tests may use various samples including: serum, milk and ear notches (Lanyon, Sasha R). Antigen- ELISA assay is expected to have a sensitivity of 100% and a specificity of 99.3% (Lanyon, Sasha R). A recent commercial release of a SNAP test for rapid, cow-side detection is readily available for detection of persistently infected animals (idexx.com). Disadvantages of using Ag-ELISAs for detection is that colostral antibodies may affect the sensitivity of the antigen assay and cross reaction with border disease virus has been reported (McF- adden).

Immunohistochemistry is by far the most recognizable and popular diagnostic approach to detection of persistently infected animals in the United States (Lanyon, Sasha R.). Immunohistochemistry is expected to have a sensitivity of 99.6% and specificity of 99.6 % (Lanyon, Sasha R.). A Cornish, 2005, study showed that immunohistochemistry can also detect acutely infected calves from ear notches. In this study, three out of eight calves tested positive for acute infection. They tested negative for RT-PCR and virus isolation on buffy coat. Immunohistochemistry can detect antigen infection in chronic cases. Immunohistochemistry although thought as ideal for large numbers of samples does pose disadvantages in collection and testing. It is very labor intensive with a need for proper facilities and handling, it relies on a subjective scoring process, and prone to technical error (Lanyon, Sasha R.). Samples cannot be prolonged for testing as they will be unreliable tests if stored in formalin for >15 days (Khan).

Antibody testing (Ab-ELISA) may be indicated to distinguish between acute and persistent infection in individuals with a positive RT-PCR result. Serial post BVDV infection antibody tests may be the most common method of detecting acute infection with rising titers

(Lanyon, Sasha R.). If BDVV leads to fetal abortion tissue samples may be taken for proper identification of infection. Fetal BVDV infection may be determined by virus isolation, IHC, Ag ELISA of fetal fluid/skin, and PCR of fetal fluid (Lanyon, Sasha R.). These tests vary in properties to identify active or persistent BVDV infections; thus, herd history and testing goals are paramount in determining the best diagnostic approach for a particular herd. Financial constraints and economic stability may also play a key role in diagnostic planning strategy for a herd with possible BVDV infection (White, Brad).

**Treatment and Management**

Diagnostic testing is implemented to detect herd status of BVDV infection. Thus, diagnostic results allow proper control strategies and management practices to concurrently take place. Test and cull schemes have been applied around the world (Lanyon, Sasha R.,). Since persistently infected animals are the primary transmission source, efforts should be directed to eliminate the formation of PI calves (White, Brad). If a herd is BVD negative, then a strong biosecurity program should be implemented. A closed herd is ideal, however, producers who purchase cattle from stockyard operations should quarantine new additions with proper screening. Protecting the pregnant cow prior to four months of gestation is the most important way to eliminate persistent infections (White, Brad). Treatment is not available for BVDV infection in a herd. Test-positive animals for PI status should receive euthanasia or segregation of individuals affected, to remove this BVDV reservoir (Larson, Robert L.). Switzerland, is now largely regarded as BVDV free, due to successful eradication schemes (Lanyon, Sasha R.,). Immunization against BVDV is the major component of prevention and control programs (White , Brad). Both killed and modified live vaccines are readily available for the prevention of BVDV (Ridpath, J.f.). Modified-live viral vaccines have proven to mount some immune response against fetal infection

with BVDV (White, Brad). Bivalent vaccines that offer coverage of both biotypes (cytopathic and noncytopathic) are available and may prove to be advantageous for cattle producers (White, Brad).

**Conclusion**

Understanding the complex pathology and pathogenesis of BVDV will guide diagnostic approaches toward identification of disease. (Lanyon, Sasha R.,). The persistently infected animal is the reservoir for disease and identification of these animals is important for long term control (White, Brad). Good biosecurity and vaccination programs aid in proper prevention of this disease. Evaluation of each cattle producers herd status (low or high risk) must be determined for implementation of an economically efficient strategy in aiding against bovine viral diarrhea virus.

## References


- Borel, N., Janett, F., Teankum, K., Zlinszky, K., Iten, C., Hilbe, M., 2007. Testicular hypoplasia in a bull persistently infected with bovine diarrhoea virus. Journal of Comparative Pathology 137, 169–173.

- Khan, F., Vorster, J.H., van Vuuren, M., Mapham, P., 2011. Evaluation of the effects of long-term storage of bovine ear notch samples on the ability of 2 diagnostic assays to identify calves persistently infected with bovine viral diarrhoea virus. Journal of the South African Veterinary Association-Tydskrif Van Die Suid- Afrikaanse Veterinere Vereniging 82, 18–23.

- Lanyon, Sasha R., Fraser I. Hill, Michael P. Reichel, and Joe Brownlie. "Bovine viral diarrhoea: Pathogenesis and diagnosis." *The Veterinary Journal* 199.2 (2014): 201-09. Web.

- Larson, Robert L. "Bovine Viral Diarrhea Virus–Associated Disease in Feedlot Cattle." *Veterinary Clinics of North America: Food Animal Practice* 31.3 (2015): 367-80. Web. Apr. 2017.

- Letellier, C., Kerkhofs, P., Wellemans, G., Vanopdenbosch, E., 1999. Detection and genotyping of bovine diarrhea virus by reverse transcription-polymerase chain amplification of the $5^0$ untranslated region. Veterinary Microbiology 64, 155– 167.

- Maurer, K., Krey, T., Moennig, V., Thiel, H.J., Rumenapf, T., 2004. CD46 is a cellular receptor for bovine viral diarrhoea virus. Journal of Virology 78, 1792–1799.

- McFadden, A., Tisdall, D., Hill, F., Otterson, P., Pulford, D., Peake, J., Finnegan, C., La Rocca, S., Kok-Mun, T., Weir, A., 2012. The first case of a bull persistently infected with Border disease virus in New Zealand. New Zealand Veterinary Journal 60, 290–296.

  Pedrera, M., Gómez-Villamandos, J.C., Risalde, M.A., Molina, V., Sanchez-Cordon, P.J., 2012. Characterisation of apoptosis pathways (intrinsic and extrinsic) in lymphoid tissues of calves inoculated with non-cytopathic bovine viral diarrhoea virus genotype 1. Journal of Comparative Pathology 146, 30–39.

- Peterhans, E., Jungi, T.W., Schweizer, M., 2003. BVDV and innate immunity. Biologicals 31, 107–111.

- Ridpath, J.f. "Bovine Viral Diarrhea Virus." *Encyclopedia of Virology* (2008): 374-80. Web. Apr. 2017.

- Ridpath, Julia F. "Bovine Viral Diarrhea Virus: Global Status." *Veterinary Clinics of North America: Food Animal Practice* 26.1 (2010): 105-21. Web.

- White, Brad. "Applied BVD Management." *Western Veterinary Conference 2006* (2006): 1-3. *VIN*. Web. Mar. 2017.