# Steer Clear of This Heifer, She's No Bull

Bovine Viral Diarrhea Virus-Induced Mucosal Disease in a Crossbred Heifer

Austin Whitmon

Mississippi State University

College of Veterinary Medicine

Class of 2020

Clinicopathologic Conference

January 17th, 2020

Advisor:

Brittany Baughman, DVM, M.S., DACVP

## Introduction

Bovine Viral Diarrhea Virus (BVDV) is an economically important disease that has plagued the cattle industry, beef and dairy alike, for many years. BVDV, a *Pestivirus* from the family *Flaviviridae,* has been a topic of research, as well as puzzlement, for over half a century since its' initial discover in the late 1950's. BVDV is complex not only in the sense that it can cause a wide array of clinical signs, but that it possesses the ability to manipulate and evade the hosts' immune system. There are two known genotypes of BVDV, BVDV-1 and BVDV-2, each further subdivided into subgenotypes. There are 17 known subgenotypes of BVDV-1 (BVDV-1a-1q) and three subgenotypes of BVDV-2 (BVDV-2a-2c).[6] Of the subgenotypes, three are responsible for most of the reported cases in North America, BVDV-1a-1b and BVDV-2a, with BVDV-1b being over represented.[6] Both genotypes are also categorized into one of two biotypes, non-cytopathic (ncp) and cytopathic (cp), depending on the effects within the host. The ability of the virus to persists within a herd is a result of fetal infection with a ncp BVDV strain between days 45-125 of gestation.[8] This occurs prior to the fetal development of immunocompetence, therefore producing a persistently infected (PI) calf, who's immune system recognizes the viral infection as "self". Mucosal disease (MD) is a rare, but highly fatal sequalae of BVDV infection, only witnessed in PI calves. MD results from the superinfection of a PI calf with a homologous cp BVDV strain or a mutation of the current infective ncp BVDV strain.[2] MD has a 100% fatality rate and is characterized by a unique set of gross anatomical findings.

## History and Presentation

Heifer 3-58 was an approximately 7-month-old Brangus-cross that presented for necropsy on October 18th, 2018. Heifer 3-58 was a part of a 137-head herd of commercial cattle, comprised of 76 cows and 61 calves, in South-Central Mississippi. The calf was born and raised

on site and at the time of presentation there was no history of new entries into the operation. On the evening of October 18th, 2018, the calf was found to be lethargic, exhibited low head carriage, and appeared to have hindlimb paresis. From the referring veterinarian's records, it was reported that upon examination the calf was in sternal recumbency, resting on her stifles with both rear hooves positioned laterally. The calf expired approximately 15-minutes after examination and was referred to the MSU-CVM Pathology service for necropsy and diagnostic testing. The only relevant history given at that time, was the calf had been vaccinated 15-days prior to death with Inforce 3 (BRSV, IBR, PI3) intranasal, Bovi-Sheild GOLD 5 (MLV; IBR, PI3, BRSV, BDVD Types 1 and 2) subcutaneously, One Shot Ultra 8 (*Clostridium chauvoei, septicum, haemolyticum, novyi, sordellii, perfringens* types B, C, and D, and *Mannheimia haemolytica* type A1) subcutaneously, and dewormed with Safe-guard (fenbendazole) orally.

Further investigation by the MSU-CVM Population Medicine Department, at the owner's request, revealed that there was a history of recent fence line contact with a neighboring herd. The herd from which this calf originated was comprised of two separate herds, recently combined. Half of the herd (Herd 1) had been maintained on the current pasture and the other half (Herd 2), had been housed on a separate pasture located a few miles away. Herd 2 had fence line contact with a neighboring herd as well as a herd bull, which at the time of the outbreak investigation had been sold due to destructive behavior, the final incident resulting in the herd bull tearing down the fence line separating Herd 2 from the neighboring herd. The bull was servicing cows from the neighboring herd for an unknown period of time. Once discovered the herd bull was sold and Herd 2 was relocated and combined with Herd 1.

It was previously stated that there had been no new entries into the operation, however upon visiting the operation there were approximately 50 steers, obtained from Florida, being

housed on a dry lot within the resident herd's pasture. There was limited fence line contact between the two groups, though contact was possible. Both sets of cattle were also being processed through the same working facilities, and there was a shared water source identified. At this time there were multiple breaches in biosecurity within the operation, most likely attributing to the infection and death of Heifer 3-58.

**Necropsy and Microscopic Findings**

Upon external examination the calf was assessed and assigned a body condition score (BCS) of 4/9. There was no fecal staining of the perineum, tail head, or rear limbs noted. The eyes were sunken bilaterally, and the nictitating membranes were mildly-hyperemic. Mild ruminal reflux was appreciated in the oral and nasal cavities. No additional external changes were observed.

Oral examination revealed multifocal to coalescing, variable sized hemorrhagic erosions and ulcers along the inner lip mucosa, the buccal and lingual mucosal surfaces, and along the hard palate and tongue. The pharyngeal mucosa was edematous, diffusely reddened, and contained multifocal, small 2 mm x 2 mm mucosal ulcers. At the level of the arytenoids there were multifocal ulcerative lesions measuring 4 mm x 6 mm. Upon opening the esophagus there were widely disseminated punctate hemorrhagic ulcers extending the length of the esophagus. Within the abdominal cavity the small intestines were gas filled with segmental areas of congestion. The rumen was distended with feed material and the abomasum was fluid filled. Within the rumen there was a large amount of feed material accompanied by a large mass of hay-string and a consumed fly-tag. The ruminal villi were partially autolyzed, blunted, and multifocally reddened. The abomasum contained about 200 mLs of a dark green odiferous fluid. The abomasal mucosa was congested diffusely and there was minimal mucosal edema. The small

intestines contained green watery luminal material and there was a focal spot of hemorrhage measuring 4 mm x 4 mm on the mucosal layer of a segment of the small intestine. There were occasional small hemorrhagic ulcers throughout the small intestines. Multifocal areas of hemorrhage within the spiral colon were noted. The distal colonic mucosa was multifocally reddened. The adrenal glands contained multifocal areas of cortical hemorrhage. Gross examination of the heart, liver, kidneys, pancreas, urinary bladder, and brain was unremarkable.

On histopathology of the tongue and oral mucosa there were multifocal superficial erosions and full thickness ulcers on the glossal and oral mucosal surfaces, surrounded by thin rims of hemorrhage. Microscopic examination of the esophageal mucosa revealed numerous discrete areas of ulceration that were often covered by amorphous eosinophilic necrotic debris intermixed with cocci and bacilli bacteria. Ulcerative foci within the esophageal mucosa were multifocally marginated by thin zones of hemorrhage and congested blood vessels. Ruminal villi were multifocally necrotic. The mucosal epithelium was multifocally ulcerated and the underlying lamina propria was expanded and infiltrated by increased numbers of intact and degenerate neutrophils and lymphocytes. Mucosal and submucosal vessels were dilated and occluded by intraluminal fibrin. Sections of small intestinal mucosa exhibited mucosal and submucosal blood vessels, that were dilated and congested with blood. In select areas of small intestinal mucosa, deep mucosal crypts were absent, and there were occasional crypt abscesses noted. Multifocal submucosal and mucosal blood vessels were dilated and occluded by intraluminal aggregates of fibrin, intermixed with inflammatory cells. Multifocal areas of vasculitis within the small intestines were present. These histopathologic lesions in combination with the clinical signs led us to the diagnosis of BVDV-induced mucosal disease.

## Diagnostic Approach

Tissue samples were collected at the time of necropsy and in-house diagnostic testing was performed. An ear notch antigen capture ELISA (ACE) was performed through the CVM laboratory and was negative. Tissue samples were then submitted to the Mississippi Veterinary Research and Diagnostic Laboratory (MVRDL) for further testing. The ACE ear notch test was repeated at the MVRDL and was positive. It was assumed at that time that the original ACE test was negative as a result of user error. Further diagnostic testing included virus isolation (VI) and polymerase-chain reaction (PCR) of splenic tissue. Neither testing modality yielded positive results. Whole herd testing was initiated after the first positive result was confirmed. Ear notch samples were submitted on the rest of the herd, and two other PI calves were identified.

## Pathophysiology

Bovine Viral Diarrhea Virus (BVDV) has been the topic of research since its' original discovery in 1946, though due to the virus' complexities it is still not fully understood.[2] BVDV possesses the ability to infect cattle of all ages leading to significant production losses, as well as reproductive insufficiencies. Eradication efforts have been implemented over the years however due to the virus' ability to evade the host immune system, inapparent shedders or persistently infected (PI) calves propagating the disease have posed obstacles.[1] PI calves have been estimated to comprise <1% of the world's cattle population, though due to their effective shedding abilities they are able to maintain BVDV within cattle herds worldwide.[7]

PI calves are the most important reservoir of BVDV and are crucial to survival of the disease. PI calves are the direct results of fetal infection with a particular BVDV biotype. BVDV is classified into one of two biotypes, cytopathic (cp) and non-cytopathic (ncp). The ncp BVDV

biotype is able to persists within the hosts without triggering an immune response, thus leading to the development of PI calves. The cp BVDV biotype however, is able to evade the hosts' immune system, and induce an extensive inflammatory response and impair anti-viral defense mechanisms.[1] Both biotypes individually are capable of their own set of clinical signs, but together they lead to a rare, and highly fatal disease manifestation known as MD.

PI cattle represent a minute portion of the world's cattle population, but effective viral spreading allows for maintenance of the virus within herds. PI cattle are a result of a ncp BVDV viral infection of the dam and fetus between days 45-125 of gestation.[3] Timing of the infection is critical to the development of PI calves as fetal immunocompetence is not developed until days 125-175 *in utero*.[8] Therefore, the infected fetus recognizes the ncp BVDV as "self", and mounts no significant immune response, allowing the virus to persist. PI calves are inapparent carriers of BVDV, circulating among the herd, and effectively transmitting the virus. Herd outbreaks are often not suspected until acute deaths or reproductive losses are identified.

BVDV infections, propagated by PI cattle, can cause acute or transient infections in susceptible herdmates. BVDV causes immunosuppression of the infected individual, increasing their susceptibility to secondary bacterial infections, respiratory pathogens being the main concern. Widespread respiratory disease can lead to increased fatalities amongst acutely infected individuals. BVDV is successfully maintained however due to the reproductive effects of the disease. The disease manifestations vary depending on the gestational age of the fetus at the time of infection with BVDV, as well as the infective biotype. Exposure of heifers or cows, to BVDV, prior to conception can negatively impact herd conception rates and lead to early embryonic losses. A BVDV infection with the ncp or cp biotype, are capable of causing abortions if the fetus becomes infected between days 45-175 of gestation.[3] Congenital defects

and malformations of the fetus are often what clue producers in to an underlying issue. A BVDV infection occuring at 100-150 days of gestation can result in a plethora of congenital abnormalities depending on the stage of organogenesis of the fetus.[6] Commonly recognized defects are cerebellar hypoplasia, hydranencephaly, microphthalmia, and brachygnathism. It is important that veterinarians be aware of the wide array of congenital defects and malformations as BVDV can spread rapidly throughout a herd, infecting dams at varying gestational ages. The most important reproductive effect however, is the development of a PI calf.

PI cattle are often associated with spreading of the virus, however PI calves are not immune to BVDV entirely. PI calves that become superinfected with a cp BVDV biotype, homologous to the infective ncp strain, fall victim to a 100% fatal disease form known as mucosal disease.[2] MD is thought to be an extremely painful disease characterized by ulcerations and erosions of the digestive tract, as well as lesions of the skin and hooves.[3] In the case of MD there are two key diagnostics that aid in the diagnosis. Firstly, isolation of the infective cp and ncp BVDV biotypes from postmortem tissue collection, and secondly confirmation of PI status via immunohistochemistry (IHC) or antigen capture ELISA (ACE).[3] As only PI cattle can exhibit MD, a negative ACE would confirm the patient was transiently infected.

PI cattle often do not survive to adulthood, either due to the induction of MD or overwhelming secondary infections. These individuals, however are the main reservoirs of all BVDV worldwide and therefore control efforts are aimed at identifying and removing PI cattle from the herd. There are many diagnostic tests available to help identify PI cattle such as VI, IHC, ACE, and PCR. The “gold standard” method of identifying PI cattle are two consecutive VI, 3-4 weeks apart.[7] However, the more commonly used testing modalities are ACE and IHC performed on ear notch biopsies. Ear notch biopsies are easily obtained and provide producers

with a readily identifiable way to differentiate tested from untested cattle.[8] Diagnostic testing is not without its' own complications as BVDV has a highly variable genome and is able to undergo genetic mutations within the hosts, which make identification difficult.[10] This means multiple diagnostic methods may be needed in order to accurately confirm the diagnosis.

**Management**

BVDV is a highly contagious and economically important disease within the cattle industry, for which there is no curative treatment. PI calves are able to infect 70-100% of susceptible herdmates when comingled or allowed fence line contact.[3] Control of the disease is achieved through biosecurity or biocontainment. Biosecurity is used in herds who have identified themselves as BVDV-free, meaning they wish to implement measures to keep BVDV out of the herd. This is achieved through testing all purchased cattle, and isolating purchases from the resident herd for a period of 3-4 weeks. If testing is not performed than recognition of the isolation period is advised. Contact of new entries with pregnant cattle from the resident herd is prohibited as a single infection could lead to catastrophic effects post-parturition. Pregnant cow/heifer purchases must be isolated from the resident herd until after parturition and testing of the delivered calf is performed. Pregnant cow/heifer purchases do not have to be tested, as a negative ACE, could mean the dam has cleared a BVDV infection. However, there is still a possibility the dam is carrying an infected PI calf. Only once confirmatory testing on the calf has been performed can the pair enter the resident herd.

Biocontainment is initiated when there has been a PI calf identified within the resident herd, and measures are taken to ensure all infected individuals are identified and disease transmission can be halted. This can prove to be an expensive endeavor initially, however unidentified PI calves within a resident herd can cost exponentially more. Whole herd testing

should be performed to identify and remove all BVDV-PI calves. Diagnostic tests such as VI, PCR, IHC, and ACE on ear notch samples are often used to detect indwelling PI calves.[8] In order to prevent effective contacts, cattle testing positive should be separated from those with negative results. Separation of all pregnant individuals from the rest of the herd is also recommended as the risk of transmission and creation of a PI calf is high. It is important to remember that pregnant cows and heifers can test negative for BVDV and still be carrying a PI calf. Testing of newborns is crucial to effective biocontainment. If acreage allows, it would also be beneficial to institute the Sandhills Calving System, where after a calving occurs, all remaining pregnant cows and heifers are then moved to another pasture. This system protects uninfected newborns from acquiring BVDV, and also prevents the possibility of a newborn BVDV-PI calf from infecting a pregnant dam.

Biosecurity and biocontainment alike require periodic surveillance testing to ensure effective removal of PI calves. An appropriate BVDV vaccination program is crucial to the disease control but should never be used as a standalone method. The goal of vaccination is to limit the spread of disease, as well as reduce the development of clinical signs amongst diseased individuals. Both killed and modified-live virus (MLV) BVDV vaccines are available in the United States, and the use of one over the other is still a major controversy. MLV vaccines are known to elicit a stronger immune response, however they are not without risks. MLV BVDV vaccines can induce disease in susceptible cows resulting in fetal infections. Vaccination of an unidentified PI calf with MLV BVDV vaccine can result in what is known as post vaccinal mucosal disease (pvMD), resulting in the same pathologic lesions as a true MD infection.[7] Often times the presence of a PI calf may go unnoticed until an incident such as this arises.

## Conclusion

Mucosal disease (MD) is a rare and extremely fatal disease that is only inducible in a small subset of cattle known as PI calves. BVDV most likely entered the herd due to a lack of biosecurity. The breach in biosecurity most likely occurred due to the fence line contact with neighboring herds or the recent addition of stocker cattle. Heifer 3-58's lesions and ELISA results support the diagnosis of MD. Although the ACE was positive, BVDV-PCR and VI were unrewarding. These negative results may be a result of mutations or antigenic alterations of the 5'-untranslated genomic region (5'-UTR) and/or the *E*2 gene of BVDV, both of which are highly variable regions of the BVDV genome that assists in the recognition of the infective BVDV genotype. A recent study has shown that genetic mutations and antigenic alterations at these sites can negatively impact the sensitivity of our diagnostic testing modalities.[10] However, one positive result on the ear notch ACE was confirmation of the PI status. Furthermore, two additional animals from this herd were confirmed to be PI calves. The rare induction of MD was a result of a superinfection with a genetically similar cp BVDV isolate. Superinfection often occurs when a PI calf is exposed to a herdmate infected with the cp BVDV biotype, however the use of MLV vaccines have been shown to induce MD as well. With the herd's recent history of vaccination with an MLV BVDV vaccine, pvMD cannot be ruled out. Regardless of the cause of induction, Heifer 3-58's death uncovered an underlying disease outbreak, and led to subsequent diagnoses of PI calves within the herd as well as initiation of biocontainment measures.

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