Discovering the Diarrhea that Runs in Her Genes: An Outbreak Investigation

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INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an immunosuppressive pathogen causing multi-systemic disease in cattle around the world.[2] The earliest reports of BVD associated disease can be traced to the 1940s when disease sweeping across western Canada causing "pyrexia, watery and bloody diarrhea, dehydration, tenesmus, tachypnea, tachycardia, drooping ears, anorexia, excessive lacrimation, nasal discharge, hypersalivation, and development of ulcers of the nares, muzzle, lips, and oral cavity mucous membranes."[3] This unknown disease was coined "X disease" and was differentiated by transmissibility to a similar disease affecting cattle in New York occurring later that same year. Olafson et. al. characterized this new disease by "leukopenia, high temperatures, salivation, nasal discharge, diarrhea, depression, anorexia, dehydration, and abortion"[14] and later named this syndrome 'virus diarrhea of cattle' in late 1946.[3] As efforts to understand its complex pathogenesis were pursued, 'virus diarrhea of cattle' became known as the bovine viral diarrhea-mucosal disease complex (BVD-MD) in the late '60s to early '70s.[3] Through continued research, the disease-causing pathogen of this complex further became known as bovine viral diarrhea virus. Identified as a Pestivirus, BVDV has been recognized as a population of antigenically similar RNA viruses within the Pestivirus genus.[5,4] Walz et.al designated this population as a 'quasi-species': a different but closely related mutant viral genome subjected to continuous competition and selection.[4] This genetic and antigenic variation does not necessarily equate a change in virulence, but does play a role in the outcome of BVDV infection. The two major BVDV strains, BVDV1 and BVDV2, are responsible for diversity in clinical disease. However, "the clinical presentation and the outcome of BVDV infection depend on numerous factors, with host influences being very important, and these include immune status, the species of host, pregnancy status and gestational age of the fetus, and

the presence of concurrent infections with other pathogens."[4] The purpose of this case study is to describe one outbreak of BVDV and the specific biocontainment strategies applies to eliminate the virus from the herd.

PATHOPHYSIOLOGY AND EPIDEMIOLOGY

Though its original name alludes to gastrointestinal disease, BVDV has evolved to be one of the most economically crippling respiratory and reproductive diseases around the world.[1,2,4] Though the virus is best perpetuated within and between herds by infecting the dam and inducing fetal immunotolerance following fetal infection, BVDV infection can manifest in a variety of clinical conditions.[2,4] Disease due to BVDV can be convoluted, and it is contingent on specific host factors (i.e. age, pregnancy status) as well as the involved viral strain. The three major clinical scenarios associated with BVDV infections include: acute infection, fetal infection, and persistent infection.[8] In acutely or transiently infected animals, BVDV infects cells of the innate and adaptive immune systems. Granulocytes, macrophages, antigen-presenting myeloid cells, and lymphocytes are all subject to invasion; however, BVDV has its major effect on lymphocytes. Through impairing cell function and inducing apoptosis, acute BVDV infection results in transient immunosuppression and provides an opportunistic scenario for secondary invaders.[7,10] This ability to induce an immunosuppressed state is critical in recently weaned calves, where BVDV induced immunosuppression becomes a pivotal role in the bovine respiratory disease complex, otherwise known as "shipping fever." Age continues to govern clinical disease in pre-weaned calves on cow-calf operations where BVDV infected calves are more susceptible to neonatal diarrhea outbreaks.[8,15] These infected, non-pregnant animals develop a viremia and begin shedding the virus 3 days post-infection and continue shedding the virus for approximately 2 weeks.[7,11] If a secondary infection or recrudescence of a latent

infection does not occur during viremia, infected animals can recover within 3 weeks. However, recovered, immune animals can carry BVDV in peripheral blood mononuclear cells for at least 98 days, threatening viral transfer to other susceptible individuals.[7] Other clinical signs associated with acute BVDV infection include diarrhea, depression, oculonasal discharge, anorexia, and oral ulcerations.[4,7]

The most important aspect of BVDV infection is its ability to create a persistently infected fetus which allows for ongoing transmission of the virus in the population. If the fetus is infected prior to the development of humoral immunity, the fetus becomes immunotolerant: both the innate and adaptive immune systems fail to recognize the virus as a foreign antigen.[9] A wide number of days have been published; however, days 45-125 of gestation are generally accepted as the window for BVDV to cause persistent infection, or PI.[4,9] Though persistently infected animals comprise less than 1% of the total cattle population, they are the primary virus reservoir within a population, shedding enormous amounts of virus into the environment directly through nasal and ocular discharges, urine, semen, colostrum/milk, and feces[4,7,12] All PI dams give birth to PI fetuses, maximizing both horizontal and vertical means of transmission.[4] Thus, one of the key features of BVDV control and prevention programs is to prevent virus exposure to dams during the 45-125d susceptibility window of gestation to prevent the creation of persistently infected animals.[4,12]

Persistently infected animals acquiring a secondary BVD virus develop mucosal disease.[5] BVDV is classified into 2 major biotypes which are characterized by their activity in cell culture: noncytopathic and cytopathic. Although limited clinically, biotypic classification is essential for understanding the development of mucosal disease. Lanyon et. al describes this process occurring as one of three mechanisms. First, and most likely: “disease is associated with the

appearance of a ‘cytopathic’ BVDV biotype arising from mutation of ‘noncytopathic’ BVDV already circulating in the PI animal.”[7] Second, PI animals can acquire a superimposed infection with an antigenically homologous cytopathic virus to the noncytopathic virus. Finally, PI animals can share their antigenically similar noncytopathic viruses and subsequently develop mucosal disease. All three scenarios lead to mucosal disease: an inevitably fatal manifestation of uncontrolled inflammation and enhanced viremia.[7]

Infection after the first trimester and later in the 45-125d window can lead to congenital defects in the fetus.[2,7] During this time, organogenesis is occurring in the fetus and the immune system is concluding maturation. Grooms et. al suspect that “the combination of direct cellular damage by virus and inflammatory responses to virus have been proposed as mechanisms.” The most common congenital deformities include: microencephaly, hydrocephalus, hydranencephaly, porencephaly, cerebellar hypoplasia, and hypomylenination, with cerebellar hypoplasia presenting as the most widely recognized.[2] Other deformities such as ocular malformations, brachygnathism, and thymus, bone, and lung growth retardation have been documented. Finally, viral infection at this gestational stage can result in fetal death and abortion.[7]

OUTBREAK INVESTIGATION

On February 3, 2018, a 2-month-old bull calf was received by the MSU-CVM Diagnostic Laboratory Service for necropsy. The history on intake stated that the producer had lost four 1-2-month-old calves the previous week. Prior to death, these calves were treated with tulathromycin (Draxxin), flunixin meglumine (Banamine), and ceftiofur crystalline free acid (Excede) for presumed respiratory disease characterized bilateral yellow nasal discharge and coughing. Necropsy findings disclosed a severe bronchopneumonia with *Pasteurella multocida*, gastrointestinal parasitism, and BVDV infection. After receiving the necropsy results, the

producer's veterinarian contacted MSU-CVM Department of Pathobiology and Population Medicine for consultation and further work-up.

On Thursday February 22, 2018, the MSU-CVM Population Medicine rotation visited the property where a herd of 90 mixed-breed beef cows and their calves are maintained on ryegrass pasture. Historically, the herd had been in good health despite the lack of a herd health program. Previously, only bulls have been introduced to the herd; no female breeding stock had ever been introduced. In mid-November 2017, the producer purchased seven bred mixed-breed beef cows from a livestock auction market. These new additions were quarantined for two weeks; however, they had nose-nose fence-line contact with most of the herd. Shortly thereafter, adult cattle in the main herd exhibited mild crusting around the eyes after the seven were introduced to the property; however, they recovered without treatment. In early January 2018, a small calf was born to one of the seven 'new' cows. This calf ultimately died and was followed by a stillbirth of another calf. In late January to early February, calves in the original herd began exhibiting signs of respiratory disease. The producer treated 14 sick calves to which 10 failed to respond to treatment, ultimately dying. This respiratory epidemic coincided with calving of the 'new' herd additions. After the return of the MSU-CVM necropsy report, all cows and calves were vaccinated with a Pastuerella/Mannheimia bacterin and all calves were treated with Nuflor as directed by the producer's regular veterinarian.

Though the necropsy report revealed severe bronchopneumonia and *Pasteurella multocida* was cultured from lung tissue, *P. multocida* was not the primary agent responsible for the collective losses that the producer experienced. The cumulative high morbidity and mortality in young, unvaccinated calves after the introduction of seven pregnant sale barn animals into a

'semi-closed' herd is a classic presentation of BVDV. BVDV was the primary agent responsible for respiratory compromise, allowing *P. multocida* to flourish as a secondary invader.

DIAGNOSTIC APPROACH/CONSIDERATIONS

The accumulation of evidence including the history, necropsy report, and ongoing clinical scenario made a BVDV diagnosis nearly irrefutable. However, in outbreak situations other than that outlined above, BVDV should be a considered for any herd suffering widespread reproductive loss and/or respiratory disease. According to Grooms et. al., electing to test for BVDV within a herd is two-fold: to identify the presence of BVDV and most importantly, to identify PI cattle. Because PI animals are the primary virus reservoir within herds, control programs target their identification and subsequent elimination. The most commonly employed test employs immunohistochemistry or antigen capture ELISA (ACE) to detect antigen in skin samples, usually via an ear notch.[2,4] Ear notching has become a widely employed screening tool because it can be used on animals of any age, and a single sample is usually all that is required for diagnosis. According to Walz et. al, "Skin biopsies are easy to obtain, and testing can be performed on young PI animals that would test negative by virus isolation, microplate virus isolation, and ACE testing on serum because of inhibition of the tests by colostral antibodies."[4] Because PI animals are the primary target of control/screening programs and because PI animals are usually younger stock, selecting a test without maternal antibody interference is valuable in enhancing the predictive value of a positive test.[11]

TREATMENT AND MANAGEMENT

Unfortunately for those affected by BVDV, no treatment is available for cattle infected with BVDV. Most management strategies for herds affected by BVDV employ a test and elimination protocol: all cattle determined to be PI for BVDV from tissue samples are culled from the herd.

However, it is important to outline an attainable goal for the producer prior to commencing any control or eradication program. A ‘one size fits all’ approach is inappropriate and a waste of resources. Lindberg and Houe describe four major elements to a successful control/eradication approach to BVDV: “Predictors of progress for systematic control approaches in general are discussed in terms of the abilities to: prevent new infections, to rapidly detect new cases of infection, to take action in infected herds, and to gain acceptance by stakeholders.”[13] The foundation of this successful control program is established on maintaining biosecurity. More specifically, protecting susceptible female stock capable of producing a persistently infected calf from BVDV infection is the epitome of a quality model for systematic BVDV control.[13] This biocontainment protocol promotes identification and elimination of PI animals which can then be followed by routine herd monitoring/surveillance and vaccination. Specific biocontainment, screening, and vaccination logistics can be coordinated pending the appropriate resources that are available to initiate the program.[4,8,13]

CASE OUTCOME

After the MSU-CVM Population Medicine Department’s consult with the producer and the herd veterinarian on February 22, 2018, a strategy to control and eradicate BVDV from the herd was initiated. Due to the economic significance of acute or endemic infection, a successful control and prevention program requires a multi-modal approach tailored to the individual producer.[4] This approach entails preventing contact between susceptible animals, removing PI animals from the herd, and boosting host immunity through vaccination.[15]

First, following the recommendations of the MSU-CVM Population Medicine Department, a herd identification system was established. Historically, animal identification and record keeping practices were not in place on the farm. Proper identification was necessary to keep calves with

unknown BVDV status separate from early gestation cows. To facilitate tracing cow-calf pairs, all cows and calves were identified with ear tags and this information was recorded. Second, all calves were ear notched, and the tissue samples were submitted for a BVDV ELISA. An initial positive test was confirmed with a duplicate test; two positive test results confirmed a PI status, and the animal was humanely euthanized. All dams of calves testing PI were also ear notched to test and eliminate PI dams. While the herd was processed for identification and ear notching, a killed Pasteurella vaccine booster was administered to induce adequate immunity and minimize losses to the current respiratory outbreak. Additionally, a killed BVDV vaccine was administered to prevent acute infections in susceptible pregnant females and mitigate fetal infection. This vaccine was boostered approximately 3 weeks later. Next, all cows were rectally palpated for pregnancy status and were separated based on stage of gestation. The herd was subsequently divided into three groups: any cow that is 5 months pregnant or less (pasture A), any cow that is past 5 months bred (pasture B), and cow/calf pairs plus bulls (pasture C), with the anticipated flow to be from A to B to C. Eventually, after all calves were to test negative, the cattle would be back in a single herd. During this time, all animals confirmed PI were humanely euthanized. Throughout this test and elimination process, groups A and C were eventually merged. The herd veterinarian continued to stage pregnancy to mitigate co-mingling of late gestation cows with calving potential with early gestation cows pending accurate staging and sufficient labor/time to quickly move late gestation cows. This time intensive approach was necessary due to the calving management of the operation. The continuous calving system employed by this producer and many other cow-calf operations provides “time and opportunity for PI exposure to breeding cattle during the critical first 125 days of gestation.”[15] In herds with a seasonal or controlled

calving season of 80 days or less, time is available to test and remove all PI calves before exposing early gestation cattle to BVDV.

Ear notching on calves born throughout the remainder of 2018 continued and the tissues were submitted for PI status. As of September 21, 2018 when the Population Medicine Department returned to follow-up with the producer, 21 PI calves had been positively identified and subsequently destroyed. Unfortunately, a miscalculation in pregnancy status occurred and a cow gave birth to a PI calf within pasture C potentially exposing pregnant, susceptible females to BVDV. This underestimation reset the testing timeline to investigate for PI calves to March 2019. At this time, all calves with the potential for PI status should have been born and will be available for testing. As of October 12, 2018, 58/90 potential calves have been born and 32 calves have been lost to either euthanasia or disease due to BVDV. Although euthanasia of calves who may appear healthy despite their BVDV status seems harsh, it has been documented that "the presence of BVDV PI cattle also affects the health and productivity of non-PI herd mates. Beef herds with one or more BVDV PI calves present before breeding had a 5% lower subsequent pregnancy rates."[15] Smith and Grotelueschen define the benefit to the producer of eliminating BVDV from a herd as, "reduced losses from death and disease, improved productivity, and greater reproductive performance." Additionally, removing PI cattle from specific herds, "may add market value to seedstock or cattle moving into other production systems such as heifer development operations or beef finishing feedyards." In herds previously containing PI cattle, it has been demonstrated that producers receive a return on their investment from strict biosecurity and biocontainment of BVDV.[15]

SUMMARY

The general principles of BVDV control and prevention can be condensed into three fundamental concepts: increase the resistance of the host to BVDV PI animals via vaccination, prevent co-mingling of animals that would effectively transmit BVDV, and to remove or prevent introduction of BDVD PI animals.[15] Though the initial investment is high, the benefit of BVDV control is not only available to the producer but is perpetuated to the industry by improving the health and productivity of the cattle industry. The veterinarian has a pivotal role in advocating not only for the health and well-being of the herd, but also in promoting good management practices for the advancement of the cattle industry.

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