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Control of Bovine Viral Diarrhea Virus in Ruminants

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Bovine viral diarrhea virus (BVDV) is a diverse group of viruses responsible for causing disease in ruminants worldwide. Since the first description of BVDV as a cause of disease, it has undergone surges and lulls in importance. Epizootics of disease caused by BVDV are described. Although naming of the virus and illness implies gastrointestinal disease in cattle, BVDV is a pathogen that affects multiple organ systems in many animal species. Infection, disease, or both have been described in cattle, sheep, goats, pigs, bison, alpacas, llamas, and white-tailed deer, among others. In 2007, the Office of International Epizootics added bovine viral diarrhea to its list of reportable diseases, but the listing is as a reportable disease of cattle rather than as a reportable disease of multiple species. Although initial descriptions of disease caused by BVDV were of digestive disease, respiratory disease and reproductive losses because of BVDV are the most important economically. BVDV uses multiple strategies to ensure survival and successful propagation in mammalian hosts, and this includes suppression of the host's immune system, transmission by various direct and indirect routes, and, perhaps most importantly, induction of persistently infected (PI) hosts that shed and transmit BVDV much more efficiently than non-PI animals. Successful control and eventual eradication of BVDV requires a multidimensional approach, involving vaccination, biosecurity, and identification of BVDV reservoirs. The following consensus statement reflects current knowledge and opinion regarding the virus, prevalence and host range, clinical manifestations, and most importantly, the control and potential for ultimate eradication of this important viral pathogen of ruminants.

Virus Description

BVDV is an enveloped, single-stranded RNA virus, and is the prototypic member of the genus *Pestivirus* within the family Flaviviridae. Currently recognized species within the *Pestivirus* genus include BVDV1, BVDV2, border disease virus, and classical swine fever virus (hog cholera virus).[1] Strains of BVDV can exist as different biotypes, which are either cytopathic (CP) or noncytopathic (NCP). The classification of biotype is independent of genotype, as there exist CP and NCP BVDV1 strains and CP and NCP BVDV2 strains. Only NCP strains of BVDV induce persistent infection.[2] CP BVDV strains are relatively rare, with NCP isolates accounting for approximately 90% of BVDV isolates at a diagnostic laboratory.[3] The NCP biotype is the source for CP strains, which arise by mutations and recombinations in the NCP strain. A 3rd biotype of BVDV, the lymphocytopathic biotype, consists of a subpopulation of NCP strains that are capable of causing CP effect in lymphocytes cultured in vitro. NCP strains that are lymphocytopathic have been associated with severe clinical disease.[4]

As BVDV is an RNA virus, genetic mutations occur readily, leading to substantial genetic, antigenic, and pathogenic variation. Because of frequent mutation in viral RNA replication, BVDV exists as a quasi-species, which are different but closely related mutant viral genomes subjected to continuous competition and selection, thus resulting in genetic and antigenic variation. Nucleotide sequence differences are the most reliable criteria for differentiation of BVDV species. The differences between BVDV species are not restricted to any 1 genomic region and are found throughout the genome[5]; however, some BVDV genomic regions are more amenable to comparison or have greater biological importance between BVDV1 and BVDV2. The 5′

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untranslated region (5′-UTR) is the most commonly used region for detection and characterization of BVDV because of highly conserved areas that are favorable to PCR amplification, but the first nonstructural protein region is unique to pestiviruses, and comparison of this region among BVDV strains is being used for characterization of putative pestivirus species.[6] Subgenotypes of BVDV are described within BVDV1 and BVDV2 species, 12 among BVDV1 viruses (BVDV1a through BVDV1l)[7] and 2 among BVDV2 viruses (BVDV2a and BVDV2b).[8] Phylogenetic survey of the 5′-UTR genomic sequences of BVDV1 and BVDV2 strains reveals a similar level of sequence variation within each species,[9] and this finding suggests that these 2 species have been evolving for a similar time span. Within the U.S. cattle population, there are 3 major subtypes, BVDV1a, BVDV1b, and BVDV2a, with the BVDV1b subtype predominating from diagnostic laboratory submissions and PI prevalence studies, accounting for 78% of persistent infections in cattle in one North American study.[10]

Prevalence and Host Range

Cattle are the natural host for BVDV, and BVDV is distributed in cattle populations throughout the world as indicated by serologic surveys. The prevalence of seropositive cattle varies among countries, and is influenced by vaccine use and management practices. Surveys in North America have indicated individual-animal seropositive rates between 40 and 90%.[11,12] Herd-level prevalence, ie, the percentage of herds with unvaccinated cattle that are seropositive to BVDV, varies from 28 to 53% depending on geographic region.[13–15] In contrast, the prevalence of PI cattle is much lower and is generally believed to be <1% of all cattle.[16] PI cattle can cluster within groups of cattle, elevating the prevalence within populations. There are no random surveys that estimate the prevalence of PI cattle in North America. Despite reduced survivability, the prevalence of PI calves arriving at feedlots in the United States is between 0.1 and 0.4%,[17–19] which is similar to the 0.17% reported for U.S. beef cow-calf operations.[16]

BVDV does not possess strict host specificity. Classically, pestivirus isolates have been assigned according to the species from which they were isolated, with most BVDV, classical swine fever virus, and border disease virus isolates being recovered from cattle, pigs, and sheep, respectively. Evidence of BVDV infection as demonstrated by the identification of serum antibodies exists in over 50 species within 7 of the 10 families of the mammalian order Artiodactyla.[20–22] Species that are susceptible to BVDV infection include cattle, pigs, sheep, goats, bison, captive and wild cervids, and Old World and New World camelids, with recent accounts of BVDV infections in alpacas and wild cervids in North America receiving much attention. Clustering of pestivirus strains among 3 host groups (domestic ruminants, camelids, deer) has been proposed; however, the implications for transmission between these clusters are unknown.[23] Identification of heterologous PI hosts might have important implications for the epidemiology of BVDV,

most importantly as these nonbovid PI animals can serve as reservoirs for BVDV.

BVDV infections have been identified in Old and New World camelids. In New World camelids, seroprevalence rates <20% have been reported in both North and South America.[24–26] In North America, highest antibody titers to BVDV were detected on farms on which PI crias were present.[26] The herd-level prevalence is 25% where crias were tested in 63 alpaca herds in the United States.[27]

Historically, seroepidemiologic and experimental infection studies suggested that New World camelids could be infected with BVDV but have few or no clinical signs of disease.[25] Reports of BVDV isolation and identification of PI alpacas have concerned the alpaca industry, and the virus is now considered an emerging pathogen of New World camelids.[28] The first description of a PI alpaca was made in Canada where a BVDV1b strain was isolated from a PI cria after natural exposure of its dam to a chronically ill cria.[29] Several cases of PI alpacas have since been reported in North America and Great Britain.[28,30–32] PI alpacas can survive for several months, but low birth weights, failure to thrive, and chronic respiratory and gastrointestinal infections occur in PI alpacas. Diagnosis of BVDV infection in PI alpacas has been made through traditional virological techniques, by RT-PCR, and through immunohistochemistry (IHC); however, these tests have not been formally validated for camelids. Similar to PI cattle, BVDV antigen is identified in many tissues of PI alpacas.[28–30] All isolates examined in North America and the United Kingdom belonged to BVDV 1b genogroup when subgenotyping was performed.[28–30] All 46 BVDV isolates from alpacas in North America were NCP BVDV1b strains[31]; furthermore, the nucleotide identity in 45 of 46 isolates was ≥99% using the highly conserved 5′-UTR genomic region. This finding suggests an association of the BVDV1b genotype with infections in North American alpacas.[31] Possible explanations for this predominance of BVDV1b strains in alpacas include introduction and intraspecific spread and maintenance of BVDV1b into North American alpaca populations or that unique BVDV1b subgenotypes are able to establish transplacental infections in alpacas.[31] When simultaneous intranasal inoculation of pregnant alpacas with 3 different BVDV strains (BVDV1b of cattle origin, BVDV 1b of alpaca origin, or BVDV2 of cattle origin) was performed, PI crias were born with only BVDV1b strains of cattle or alpaca origin, but not BVDV2,[33] providing further support for a unique role of BVDV1b in alpacas. Both species of BVDV were isolated from Chilean alpacas and llamas, contrasting findings from North America and Great Britain.[34] Viremia, nasal shedding, and seroconversion were observed when alpacas were inoculated with BVDV1b or BVDV2 strains.[35] Irrespective of BVDV genotypes, biosecurity and surveillance principles are important for BVDV control in alpacas, as movement of alpacas, including dams with crias, between farms for breeding purposes is associated with reproductive disease and birth of PI offspring.[27,29,30]

Some wildlife are serologically positive to BVDV, and the virus has been isolated from individual animals. The

livestock-wildlife interface is of great concern for a number of infectious diseases, including classical swine fever virus, but less is known about the role of wildlife in the epidemiology of BVDV. Wildlife can become infected with BVDV, but other factors, including shedding of the virus, intrapopulation maintenance, and amount of interspecies contact might influence the establishment of BVDV wildlife reservoirs. Similar to cattle, PI wildlife are likely a central factor in the establishment of wildlife reservoirs, and PI animals have been identified in free-ranging and captive species. PI animals were detected among free-ranging eland (*Taurotragus oryx*) in Zimbabwe and white-tailed deer (*Odocoileus virginianus*) in the United States.[36–38] Apparent prevalence rates of persistent infections in U.S. cervid populations are 0.2% in Alabama, 0.03% in Colorado, and 0.3% in Indiana.[36–38] Whether the source for BVDV infection in these populations is contact with cattle, or the result of an endemic cycle is unknown, but evidence for both hypotheses exists, and both explanations are not mutually exclusive. Although 1 study did not identify a correlation between cattle stocking densities and BVDV seroprevalence rates in wildlife,[39] seroprevalence rates in white-tailed deer are higher on ranches where cattle were present.[40] Also, the management of cattle could have an important impact on interspecific transmission of BVDV, as there is likely less wildlife contact with housed dairy cattle compared with beef cattle in pastures.[41] Endemic presence of BVDV is indicated by seroprevalence rates exceeding 60% of caribou (*Rangifer tarandus*) that had no contact to cattle, and 60% of a mule deer population in Wyoming.[22,42] In a group of captive white-tailed deer, BVDV was maintained by exposure of pregnant does to a PI fawn, resulting in birth of PI offspring.[43] Vertical transmission of BVDV by transplacental infection resulted in continued birth of PI animals in a maternal lineage of lesser Malayan mouse deer in a zoological collection, emphasizing the potential for maintenance of BVDV in wildlife.[44]

White-tailed deer are the most abundant free-ranging ruminants in North America. Contact between white-tailed deer and cattle can occur in a typical North American pastoral setting, and this species has potential to be a reservoir for BVDV. Infections of white-tailed deer with BVDV occur by experimental and natural exposure.[45,46] Similar to cattle, the most dramatic effects of BVDV infections in white-tailed deer are fetal resorption, fetal mummification, stillbirth, and abortion.[46,47] Nasal or rectal shedding occurs in acutely infected and PI white-tailed deer, and results in transmission to other white-tailed deer.[43,48] In contrast to transmission of BVDV among white-tailed deer, spill-back infections, or infection of cattle as a result of exposure to white-tailed deer has not yet been demonstrated, but because of its importance, warrants further evaluation.

The discovery of novel pestiviruses in wildlife species might lead to new classifications within the genus Pestivirus.[49] An isolate from a giraffe is different from pestiviruses of domestic species, based on comparison of complete genomic sequences and palindromic nucleotide substitutions in the 5′-UTR.[50] A pestivirus isolated from an immature blind pronghorn is highly divergent from other pestiviruses.[51]

Although BVDV is not considered a human pathogen, its highly mutable nature, ability to replicate in human cell lines,[52] similarity to human hepatitis C virus,[53] and isolation from 2 clinically healthy people,[54,55] a Crohn's disease patient,[56] and feces of children under 2 years old who had gastroenteritis[54,55,57] create some concern regarding zoonotic potential.

Clinical Disease Syndromes and Pathogenesis

A wide range of clinical manifestations from subclinical to fatal disease occur in association with BVDV infection. 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. Viral factors influence clinical presentation and these include biotypic variation, genotypic variation, and antigenic diversity, but it is important to note that BVDV1 and BVDV2 strains can be involved in the entire spectrum of clinical disease.

Acute (Transient or Primary) Infections

The terms "acute," "transient," and "primary" have been interchangeably used to describe BVDV infection in postnatal cattle, with the ability to respond immunologically to BVDV. The source of most acute infections is cattle PI with BVDV, although acutely infected cattle can be a source of virus to other susceptible cattle.[58] The most effective route of transmission appears to be nose-to-nose contact. The majority of BVDV infections in immunocompetent and seronegative cattle are subclinical; however, truly benign BVDV infections probably do not exist, as cattle undergoing an "inapparent" infection could exhibit mild fever, leukopenia, anorexia, and decrease in milk production if observed closely. Moreover, if the infected animal is pregnant, deleterious effects can occur in the fetus. Acute BVDV infections result in signs that include diarrhea, depression, oculonasal discharge, anorexia, decreased milk production, oral ulcerations, and pyrexia, with laboratory findings including leukopenia characterized by lymphopenia and neutropenia. Peracute BVDV infections originally described in Canada and the United States result in severe clinical disease manifestations and higher than expected case fatality rates.[59] Genomic analysis of BVDV isolates from infected cattle from these outbreaks indicated the BVDV2 genotype, and this ultimately raised a renewed interest in acute BVDV infections.[9,59]

Another clinical disease manifestation in cattle acutely infected with BVDV is the hemorrhagic syndrome, which is characterized by thrombocytopenia.[60,61] The first descriptions of hemorrhagic syndrome included both calves and adult cattle naturally infected with BVDV, with severe depressions in platelet count.[60] Clinical manifestations of the hemorrhagic syndrome are primarily related to

thrombocytopenia and include bloody diarrhea, epistaxis, petechial hemorrhages, ecchymotic hemorrhages, and bleeding from injection sites or insect bites. Thrombocytopenic BVDV infections have been experimentally reproduced, almost exclusively with NCP BVDV2 strains.[61,62] Platelet dysfunction has also been described with experimental BVDV infection,[63] thus quantitative and qualitative platelet defects contribute to the hemorrhagic diathesis observed in infected cattle. BVDV infection of bone marrow megakaryocytes is important in the etiology of BVDV-induced thrombocytopenia.[62,64]

BVDV is lymphotrophic, and acutely infected cattle are immunosuppressed as a result of reduction in circulating immune cells and diminished function of immune cells. The consequence of immunosuppression is an increased susceptibility to other infectious disease agents, and the bovine respiratory disease complex is an example where BVDV plays an important role in polymicrobial disease. Cells of both the innate and adaptive immune responses are affected by BVDV. Leukopenia occurs in most acutely infected cattle, but the severity of leukopenia can be influenced by BVDV strain. Decreases in total leukocyte count and in leukocyte subpopulations appear to be less dramatic in calves experimentally infected with BVDV1 strains than with some BVDV2 strains; however, this can be simply because of the selection of BVDV strains chosen for study.[62,65,66] In general, highly virulent strains of BVDV induce greater declines in the white blood cell count than less virulent strains. Lymphopenia (T-lymphocytes and B-lymphocytes) and neutropenia are the major hematologic abnormalities.[66,67] Removal of BVDV-infected leukocytes by the immune system, destruction of immune cells by BVDV, and increased trafficking of immune cells into tissue sites of viral replication are all responsible for leukopenia. During acute infection, lymphoid depletion in the thymus, spleen, lymph nodes, and gut-associated lymphoid tissues (Peyer's patches), and the severity might also be strain dependent.[62] Diminished function in immune system cells has also been described during acute BVDV infection, and affected cells include lymphocytes, neutrophils, and monocytes and macrophages.[68–70]

Reproductive Tract Infections

The importance of BVDV on the male reproductive tract has not received the attention equivalent to the effects on female reproduction. Bulls infected with BVDV are capable of shedding virus in semen.[71–73] The virus can survive cryopreservation and processing of semen for artificial insemination.[74] Although acutely infected bulls shed lower concentrations of BVDV in semen than PI bulls, infection of artificially inseminated heifers can result from insemination with semen collected from acutely infected bulls before seroconversion.[72] Acute BVDV infections generally result in a transient viremia with subsequent clearance of the virus by the host immune system; however, prolonged infection of testicular tissue has been described under both natural and experimental conditions.[71,73] Prolonged testicular infection with BVDV was first identified in the testes of a seropositive, nonviremic bull at an artificial insemination center.[73] This bull continuously shed infectious BVDV in semen throughout his life despite the absence of a viremia and the presence of consistently high concentrations of circulating serum antibodies that neutralized the specific viral strain that was persistently shed in the semen.[75] Localized, prolonged testicular infections with BVDV have also been experimentally reproduced after acute infection of peripubertal bulls with BVDV. Viral RNA has been detected in semen for 2.75 years after BVDV exposure, and infectious virus grown from testicular tissue has been detected up to 12.5 months after BVDV exposure.[71] Protection from a systemic immune response because of a blood-testes barrier is believed to be the mechanism for the localized, prolonged testicular infection. Uncertainty currently exists regarding whether bulls with a prolonged testicular infection can become viremic and infectious to other animals.

Infection of pregnant cattle with BVDV can result in transplacental transmission and infection of the developing fetus. The economic damage caused by BVDV in susceptible breeding herds is mainly associated with the outcomes of intrauterine infections, which are dependent upon 3 main factors: (1) gestational age of the fetus at the time of infection; (2) organ system involved in the infection; and (3) biotype, virulence, and target cell range of the virus. Besides persistent infection, other outcomes of reproductive tract infections include abortion, embryonic or fetal resorption manifesting as repeat breeding, congenitally malformed offspring, mummification, and congenital infections manifesting as normal calves or calves of poor vigor. Although embryonic/fetal death and abortion are most common during the first trimester, mid- and late-term abortions and stillbirths can be caused by BVDV.[76] Congenital malformations are produced by BVDV infection between days 100 and 150 of gestation, and include cerebellar hypoplasia, hypomyelinogenesis, hydranencephaly, alopecia, cataracts, optic neuritis, brachygnathism, hydrocephalus, microencephaly, thymic aplasia, hypotrichosis, pulmonary hypoplasia, and growth retardation.[76]

The ability of BVDV to cause early embryonic death has been somewhat controversial. Infection of cattle before insemination reduces conception rates.[77] This could be due in part to ovarian infection and dysfunction as a result of BVDV viremia. Oophoritis and the presence of viral antigen in ovarian tissue occur in cattle acutely infected with BVDV.[78,79] Conception and pregnancy rates are lower if the animals are viremic at the time of insemination. Cattle viremic with NCP BVDV at the time of insemination had a 44% conception rate as compared with 79% for the control animals.[77] Further field studies have supported this theory that BVDV is involved in early embryonic death and repeat breeding syndrome.

Persistent Infection

Persistent infection is considered by many the most important aspect of BVDV infection as this is the key mode by which the virus maintains and perpetuates itself in the cattle population. Additionally, developments in diagnostic assays have focused on identification of PI

cattle, and a central component of BVDV control is the identification and elimination of this major reservoir of the virus. PI calves are the result of in utero BVDV infection during the period of fetal development from gestation day 45 to gestation day 125, which is the gestational period bracketed by the end of the embryonic stage and the development of fetal immunocompetence. Biological variation is clearly apparent regarding the gestational age at which developing bovine fetuses become immunocompetent, and it is important to note that day 125 is not an absolute date for immune system competence.[80] Biotypic variation is important, and whereas infection with either biotype is capable of causing fetal death, only NCP strains are associated with persistent infection.[2] To our knowledge, all genotypes and subgenotypes appear to be capable of causing PIs. Persistent BVDV infection appears to arise from specific B- and T-lymphocyte immunotolerance. Immunotolerance is specific to the infecting NCP strain of BVDV, and postnatal PI animals can respond immunologically to heterologous strains of BVDV.[81] For this reason, PI animals can be seropositive to BVDV, and seropositive status cannot be utilized diagnostically to rule out persistent infection. Virus is found in many tissues in PI animals and shed from multiple sites, including nasal and ocular discharges, urine, semen, colostrum/milk, and feces, thus making PI animals efficient transmitters. Vertical transmission rate is 100% as all PI cows will give birth to PI offspring. Most PI calves are born weak, stunted, and die shortly after birth or fall behind their cohorts as they mature, but some PI calves are born without observable abnormalities and are impossible to distinguish phenotypically from cohorts. PI animals can have an impaired immune response, making them more susceptible to opportunistic pathogens, and this could contribute to early death. Regardless of the clinical outcome, the true importance of PIs is the fact that they shed large amounts of virus thus serving as the major source of virus spread both within and between farms.

Mucosal disease is the most dramatic form of BVDV-associated clinical disease because of the severity and characteristics of lesions. Mucosal disease occurs when PI cattle become superinfected with a CP BVDV.[82] Because PI cattle comprise <1% of the cattle population, mucosal disease is characterized by a low case attack rate but high case fatality rate. The origin of the CP BVDV can be external, such as modified-live virus vaccines containing CP BVDV, or internal as the result of mutations of the NCP BVDV (the PI biotype) resulting in CP BVDV.[83] Cohorts of PIs that originate from the same strain of BVDV often succumb to mucosal disease in a narrow window of time. This occurs when 1 PI develops a mutation of the NCP BVDV resulting in a CP BVDV, which is then subsequently spread to PI cohorts. Multiple clinical forms of mucosal disease exist and can be divided into acute fatal mucosal disease, chronic mucosal disease, chronic mucosal disease with recovery, and delayed onset mucosal disease.[84] The clinical variations of mucosal disease are attributable to the antigenic relationship between the PI NCP strain and the superinfecting CP strain.[84]

Diagnosis

Many diagnostic tests are available for BVDV detection, and the choice of test depends on the clinical problem, the local availability of tests, and financial considerations. The majority of diagnostic tests developed are used to identify PI animals. Accurate diagnosis of BVDV infection relies upon laboratory testing, and once an accurate, positive diagnosis is made, further losses are prevented by implementation of rational management decisions and control procedures.

Isolation of BVDV in cell cultures using validated methodology is the gold standard for diagnosis of BVDV infection,[85] but because of the greater expense and time taken to report a result for this method, antigen detection or nucleic acid detection has largely replaced virus isolation for diagnosis of BVDV infection. The virus can be cultured and isolated from a variety of samples including serum, whole blood, semen, nasal swabs, and various tissues. Buffy coat cells from whole blood are the preferred sample for antemortem diagnosis, whereas lymphoid organ-related tissues are preferred samples from necropsies.[86] A microtiter virus isolation (immunoperoxidase monolayer assay) has been developed and utilized as a herd screening virus isolation assay, primarily for the detection of PI animals.[87] This test is not recommended for detecting acute infections, nor is it advisable to use this assay for testing calves <3 months of age, as passively derived colostral antibodies interfere with the test.

Antigen detection methods such as IHC and antigen capture ELISA (ACE) are used for BVDV detection largely because they provide rapid and inexpensive detection when compared with virus isolation. Additionally, results from antigen detection-based tests are highly reproducible between laboratories. The IHC and ACE tests performed on skin samples have become widely used and applied for the detection of PI cattle.[88] 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 acquired colostral antibodies.[89] The IHC and ACE tests are ideally suited for the detection of PI animals. Although these tests do not detect acutely infected cattle,[90–92] a single report indicates positive results might be observed for acutely infected cattle.[93] Whereas IHC is performed using monoclonal antibodies that detect an epitope which is not destroyed by formalin fixation and the U.S.-licensed antigen-capture ELISA kit also uses a monoclonal antibody detecting the same antigenic epitope, clinicians should note that a strain of BVDV has been detected in the United States that is not detected by conventional IHC and ACE tests.[94]

Molecular techniques for diagnosis of BVDV infection have gained widespread use as a routine diagnostic method.[88] Development of commercial kits, with rapid and simple viral RNA extraction techniques, has made molecular techniques ideal for detection of viral genomic nucleic acids. The RT-PCR assay is specific and can detect from 10^1- to 10^4-fold lower concentrations of virus than virus isolation, thus making RT-PCR more sensitive

than virus isolation.[95] The high sensitivity of RT-PCR has allowed it to be adapted for pooled testing of tissues, whole blood, serum, or milk samples, making this an economical way to detect BVDV infection in herd screening strategies. Pooling of samples and testing by RT-PCR is controversial, and if RT-PCR pooling protocols are not validated and continually assessed, their value in BVDV control programs might be counterproductive. Clinicians should also note that detection of viral RNA does not always equate to detection of infectious virus.[96]

Serologic testing can also be used to demonstrate BVDV infection but there is difficulty in differentiating antibodies produced in response to a natural infection, after vaccination, or as a result of transfer of maternal antibodies from dam to offspring. Serologic testing can be used to assess vaccine efficacy and vaccine protocol compliance, and by testing of sentinel animals to determine if BVDV exposure has occurred in the herd.[97] The serum virus neutralization test is the most commonly used serologic assay to determine BVDV specific antibody titers. This test can be used for the detection of antibodies against BVDV1 or BVDV2 strains depending upon the reference viral strain used in the test. However, there are no universally accepted reference strains for the VN test, which makes interpretation of results obtained from different laboratories difficult.

Prevention and Control

Eliminating pathogen reservoirs and limiting transmission from infected individuals to susceptible animals are the major principles for infectious disease control. PI cattle are the major reservoir of BVDV, although transiently infected animals can, to a lesser extent, also serve as a reservoir. Therefore, prevention or elimination of PIs is central to BVDV control. Development and implementation of herd health programs that limit exposure of pregnant cattle to BVDV are important for successful control. When developing a BVDV prevention and control program, 3 aspects should be considered: (1) identification and elimination of PI animals, (2) enhancing immunity through vaccination, and (3) implementing biosecurity measures to prevent BVDV exposure of susceptible cattle. Each of these three principles has been applied to BVDV control and greater success can be expected when used simultaneously in BVDV control programs.[98,99] Several European countries have successful eradication programs,[100–102] and this has encouraged veterinary and cattlemen's organizations in the United States to adopt control strategies.[103]

Identification and Elimination of PI Cattle

The major source for BVDV transmission is cattle PI with BVDV. Removal of PI animals should occur before their entry into breeding herds. This can be more easily achieved in beef cow-calf operations that follow a controlled breeding season. In this situation, all calves, replacement heifers, bulls, and nonpregnant cows without calves should be tested for PI status before entry of the bull.[104] Because PI cows always produce PI calves, a

negative test result of a calf indicates a negative PI status for the dam.[105] Dams of test-positive calves need to be tested for PI status. Most PI calves result from acute infection of their dam, so dams that test negative could re-enter the breeding herd. If pregnant cattle are present at the time of testing in herds with a controlled breeding season, they should be segregated and their calves be tested before return to the breeding herd. In herds without a controlled breeding season, young calves should be tested and removed as soon as possible to avoid transmission to the breeding herd. Screening young calves for PI status is best accomplished by PCR, ACE, or IHC on skin samples. The use of skin samples for testing young calves is advantageous in that sample collection is simple, samples can be taken from calves that have maternal antibodies, and a single positive test usually indicates PI status. Because the occasional acutely infected animal might be PCR, IHC, or ACE positive,[93] valuable cattle should be retested after 30 days using virus isolation or RT-PCR assays on blood samples.

Screening all individuals of a herd is very costly and other strategies can be more cost-effective. These strategies include evaluation of production records, BVDV evaluation of aborted fetuses, use of sentinel animals, pooling strategies by RT-PCR testing, and BVDV testing on sick or dead cattle.[104] Monitoring breeding records, calf morbidity and mortality rates, and weaning proportions are considered the minimal level of surveillance and are the least expensive, but this level of surveillance lacks sensitivity in detecting a PI animal.[16,104] As an example of the difficulty in utilizing clinical suspicion as a reason to perform herd testing, BVDV was isolated from cattle in 53% of herds where there was no suspicion of the infection,[106] and BVDV PI animals were not identified in 81% of herds where veterinarians suspected BVDV was present.[16] On the other hand, identifying BVDV in sick or dead animals, or in aborted fetuses provides the justification for further whole-herd testing for BVDV PI animals.

Because of high sensitivity, RT-PCR assays using pooled samples have been developed to screen herds for PI animals.[107] Pooled samples of serum, whole blood, bulk tank milk, and skin have been utilized in RT-PCR assays.[95,107–110] Pooled sample testing by RT-PCR is rapid and cost-effective for screening populations of cattle for PI animals. However, failed attempts to replicate this work in multiple labs indicate the sensitivity of the assay to changes in sample handling or operator variability. Subsequent testing of individuals within the positive pools can be performed by IHC, ACE, virus isolation, or RT-PCR methods.

Vaccination

Many vaccines or vaccine combinations are available for BVDV, and the majority of these USDA licensed vaccines contain BVDV in combination with other bovine respiratory and reproductive pathogens. In the past, most BVDV vaccines contained only BVDV1 strains, but because of antigenic diversity, modified-live and inactivated vaccines containing both BVDV1 and BVDV2 strains are now widely available. There are advantages

and disadvantages to use of BVDV modified-live viral vaccines and inactivated vaccines.[111] One disadvantage of inactivated BVDV vaccines is that two doses are required for the initial immunization, and a major problem with programs using inactivated vaccines is the widespread lack of compliance among producers by failing to booster the primary series.[112]

Vaccines are an important component to BVDV prevention, and their effectiveness has been to limit transmission and clinical disease rather than completely prevent infections with BVDV, as has been demonstrated in experimental and field studies using either inactivated or modified-live BVDV vaccines.[59,113] Protection from clinical disease is important for stocker/backgrounder and feedlot operations, and cattle that arrive at a feedlot with antibody titers to BVDV tend to have protective immunity against bovine respiratory disease complex.[114–116] Preconditioning cattle by preweaning and vaccinating against BVDV and other respiratory pathogens before commingling and shipping reduces the incidence of bovine respiratory disease in feedlot cattle.[115]

Vaccination against BVDV should protect against viremia and prevent dissemination of virus throughout the host, including preventing infection of the reproductive tract and fetus. The focus for vaccine efficacy has shifted from protection against clinical disease to protection against fetal infection. Protection against fetal infections after BVDV vaccination varies, being influenced by use of inactivated or modified-live vaccine, the timing of challenge, and the degree of homology between vaccine and challenge strains. Fetal protection is superior when animals are challenged with strains from the same genotype. Although protection is not 100%, the level of protection is superior to that observed when proper vaccination is not utilized as evidenced by higher rates of PI animals in unvaccinated cattle.

Biosecurity

After the elimination of PI animals, strict biosecurity is essential to prevent reintroduction of the virus. All purchased cattle should be isolated and tested for PI status before entry into the herd. Isolation of new additions for 3 weeks before entry into the resident herd should prevent transmission of BVDV from acutely infected animals. Most lapses in herd biosecurity involve purchasing PI cattle or purchasing pregnant cattle with unknown BVDV status of the fetus. Purchased pregnant cattle should be isolated and their offspring tested to ensure that they are free of BVDV. Semen should only be used from bulls that have been tested for BVDV infection. For purebred herds marketing valuable embryos and livestock, testing of embryo transplantation recipients for PI status is essential. Exposure of cattle to other ruminants at exhibitions should be limited, and animals should be quarantined for 3 weeks before reentry into the breeding herd. Most biosecurity principles instituted for BVDV control will benefit disease control of other pathogens. Further biosecurity principles include elimination of fence-line contact with neighboring livestock and sanitation of equipment and people entering the farm.

Outline of a BVDV Control Program

Since the discovery of BVDV, control programs have been developed and successfully implemented at the herd level. These control measures need to be multidimensional and cannot rely on 1 aspect, such as vaccination. Therefore, BVDV control requires a comprehensive programmed approach that begins with first understanding the virus, its associated clinical presentations and how it might affect the livestock industry. Producers with this understanding are better able to analyze risks and make more informed decisions. Second, it involves setting goals related to BVDV control that can be different for every operation. By understanding individual operation goals and risk tolerance, a control program can be effectively designed. Goals range from eliminating BVDV from a herd with an existing problem to keeping the virus from entering a BVDV free herd. Achieving these 2 goals will require very different diagnostic testing, vaccination, and biosecurity plans. Therefore, goals should be determined using information about the herd BVDV status, current management practices, and the likelihood of future introduction of the virus (based on animal movement and biosecurity practices). If the herd BVDV status is unknown, a strategy of serologic or virologic testing to determine the presence of the virus can optimize the control program. Measurable outcomes need to be established to evaluate progress toward goals. Objective criteria such as performance measures, reproduction data, number of health problems, or number of BVDV positive animals can be used to gauge the changes the control program has made. Accurate records provide information on the long-term viability of the control program.

Development of effective control plans requires that producers understand risks and the cost to reduce them through a risk analysis. Risks are defined by a probability of occurrence and a magnitude of loss associated with that occurrence. The magnitude of loss is termed the impact. Either part of the risk equation can be decreased through management. There is a probability that BVDV will be introduced to the herd and there is the impact of disease if it is introduced. There are also costs associated with strategies implemented to decrease the probability or impact.

Once goals are set and risk is understood, effective control strategies can be implemented. Initially, producers should be encouraged to determine if BVDV is circulating in their herd. Methods to answer this question vary in cost and reliability. Most importantly, PI animals need to be identified and eliminated. If BVDV is detected in the herd, then biocontainment protocols to minimize the negative impact of infection or eliminate circulating virus on the farm should be implemented. If BVDV is not present in the herd, appropriate biosecurity protocols to keep the herd free of BVDV should be in place.

BVDV Eradication

Many European countries have initiated BVDV control or eradication programs, with several Scandinavian

countries achieving near elimination of BVDV in their cattle population.[100–102,117] The European perspective has provided evidence that BVDV can be successfully controlled and potentially eradicated; however, designing an eradication program for 1 part of the world might not apply to other geographic regions. Control or eradication programs should be carefully constructed with information on virus characteristics and producer management practices incorporated into the program design. A key component for success of a program is level of producer compliance and program funding. Predominant type of cattle production unit, density of animal populations, amount of animal movement, and potential for contact with wildlife reservoirs are other nonviral factors that can influence implementation and success of a BVDV control program. Variation among circulating BVDV strains and vaccine usage in the region could also impact success of control. All factors need to be considered carefully if time and investment are put toward BVDV eradication in countries or regions outside of Europe. In North America, veterinary and producer organizations have formulated and/or adopted position statements on BVDV for control and eventual eradication of the virus in North America.[103] Multiple states have initiated voluntary BVDV control programs, and at present it is too early to determine their effect.

Summary and Future Directions

Considerable advancements have been made regarding our understanding of BVDV, its associated diseases, and the methods for control; yet BVDV infections remain a source for economic losses in the cattle industries worldwide. Genetic and antigenic diversity of BVDV strains, potential for nonbovine reservoir hosts, and limitations in vaccine efficacy and diagnostic accuracy are immediate and future areas of concern as control and eradication efforts are begun. Equally important to virus attributes are producer willingness and compliance with control and eradication programs, thus education efforts are imperative. Advances achieved through research have led to improved and expanded testing strategies aimed at the detection and removal of PI animals, and this has contributed to the increasing number of regional control programs. Removal of PI animals is the cornerstone for BVDV control and eradication, but enhancing herd immunity and implementing reasonable and sound biosecurity practices are important for ultimate success. Veterinarians are in a unique position to significantly impact the goal of controlling and eventually eradicating BVDV. Their broad knowledge and training provide them with the best tools to help the cattle industry make significant strides toward meeting these goals.

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