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CHAPTER 42

Viral Diseases of the Bovine Respiratory Tract

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Viral infections of the bovine respiratory tract represent significant pathogens. These infections are manifested by various clinical signs and lesions in bovine respiratory disease (BRD) with varying morbidity; mortality; loss of production (treatment costs, reduced weight gain, and carcass value); and lowered economic return to the producer. The principal viruses in BRD have historically and by emphasis on vaccination centered on bovine herpesvirus-1 (also referred to as *infectious bovine rhinotracheitis virus* [IBRV], *parainfluenza-3 virus* (PI-3V), *bovine respiratory syncytial virus* (BRSV), and *bovine viral diarrhea virus* (BVDV).[1-12] Also, bovine adenovirus (BAV) and, more recently, bovine coronaviruses (BCV) have been included.[6,13-15]

Viruses in BRD may cause primary infection with disease, either singly or in combination with other viruses. A significant role for viruses in BRD is their interaction with bacteria and *Mycoplasma* spp. in bacterial pneumonias.[8-12] These severe bacterial pneumonias are caused by *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*. *Mycoplasma bovis* represents another agent observed in these severe bacterial pneumonias often initiated by primary viral infections.

Mechanisms by which primary viral infections compromise the host, allowing for severe bacterial pneumonias, are fourfold: (1) upper respiratory tract damage to nasal mucosal epithelial cells and altered mucociliary clearance, as well as bacterial attachment, growth, and colonization; (2) tracheal mucosal epithelial cell damage reducing effectiveness of the mucociliary apparatus, compromising clearance resulting in bacterial attachment, growth, and colonization; (3) innate defenses of the airways and lung are suppressed by viral infections through damage or depletion of macrophages and neutrophils (major phagocytic cells in host defense); and (4) acquired immune system effectors such as the T-cell (cell mediated) and B-cell (humoral) suppression. These immunosuppression effects on the T-cell and B-cell systems are major risk factors caused by selected viruses with BVDV as a prime example.

Bovine respiratory tract infections occur in most types of cattle operations: postweaned beef calves going to stocker operations for forage or to feedlots directly; feeder cattle, often after grazing forage to feedlots; and dairy calves. BRD with infectious etiologies is more often observed in young rather than adult cattle. On occasion, adult cattle with BHV-1 or BRSV disease are reported.

The viruses, except for BHV-1 and BVDV, are primarily surface infections of the epithelial cells throughout the respiratory tract from the nasopharyngeal mucosa to the lungs. BHV-1 and BVDV are often associated with systemic spread of the virus, as manifested by fetal infections in susceptible females. Young calves are especially susceptible to viral infections because their maternally derived immunity from colostrum is reduced with age.[16] Calves held under stressful conditions such as markets, commingling during marketing and shipment, inadequate nutrition, overcrowding, and severe climatic changes are more prone to BRD. Often calves fresh from closed herds of the ranch operation are highly susceptible as they enter the marketing channels and are commingled with other calves, facilitating the spread of the viruses. Viruses are shed primarily in respiratory secretions of the nose, eyes, and sometimes feces. Direct or close contact with animals' infectious secretions are major modes of transmission. The morbidity rate and mortality rate (case fatality) are often low but can be much higher depending on the bacterial agents such as *M. haemolytica, P. multocida, H. somni,* or *Mycoplasma* spp. *(M. bovis)*.

The diagnosis of specific etiologic agents requires the use of diagnostic laboratory tests.[7,17] Gross lesions suggest certain etiologies; however, multiple agents may produce similar lesions. In addition, polymicrobial infections may produce the same or similar sets of lesions in affected cattle. Microscopic lesions observed histopathologically sometimes provide strong indications for an agent or perhaps families of viruses. For example, intranuclear inclusions are found in *alphaherpesviruses* such as BHV-1 and intracytoplasmic inclusions are found in paramyxoviruses such as PI-3V. However, absence of these inclusions does not rule out those agents because the inclusions may be minimal in number and tissues submitted may be inappropriate to represent viral tropism.

The clinician should consult with the diagnostic laboratory staff for the tissue submission relative to the clinical syndrome, as well as available tests. It is important that tissue sample collection and shipment be done after discussions with laboratory personnel. Certain tissues and samples require selected conditions such as freezing, fixatives, and collection with anticoagulant for blood cells. Current shipping regulations for formalin must be observed. Adequate identification of samples and submission forms assists diagnostics laboratory personnel by providing recorded history and case records. In cases with potential legal implications, recording animal ID and

vaccine serial numbers with expiration dates is useful. Likewise, a precise history of antimicrobial use including dates, dosage used (mg/kg), route of administration, and date/time of last dose should be recorded, especially when culture and antimicrobial susceptibilities are requested. The clinician should also be aware of the appropriate state laboratory, if not the veterinary diagnostic laboratory, providing rabies testing. Ideally and, if feasible, the animal to be examined could be submitted directly to the veterinary diagnostic laboratory. Often collecting samples from multiple animals or submitting multiple animals provides additional information to determine the etiology of involved agents.

VIRUS ISOLATION

Viral isolations in cell cultures are time consuming and often financially expensive to perform. Sample collection and shipment conditions such as freezing are important. Multiple passages (two to three passes) may be required. A limiting factor is the available cell lines in which the viruses are isolated. Some viruses require specific cell lines as evidenced by bovine coronaviruses.[6,15] BRSV isolation by cell culture is difficult, with reduced success associated with freezing and shipment. Ideally the viruses should cause a visible cytopathic effect (CPE) in cell culture somewhat unique for the viral family. Yet common viruses such as most BVDVs (>90%) are noncytopathic (NCP), yielding no visible cytopathologic changes in cell culture. The agent preliminarily identified in cell culture requires confirmation by neutralization with monospecific antiserum/monoclonal antibody or primary binding assays with antiserums/monoclonal antibody such as immunoperoxidase, immunohistochemistry (IHC), enzyme-linked immunosorbent assays (ELISA), fluorescent antibody assays, or immunoelectromicroscopy.

ELECTRON MICROSCOPY

Nasal swabs and fecal samples are often examined by electron microscopy. Viral families may have unique morphologies indicating a viral family. However, confirmatory tests such as immunoelectromicroscopy, as cited earlier, are required to identify the specific agent.

VIRAL ANTIGEN TESTS

Immunofluorescence has been used for several years by the diagnostic laboratory, particularly when monospecific antiserums and proper controls are used. These tests are performed on fresh tissues. More recently the use of immunohistochemistry (IHC) has largely replaced immunofluorescence. The IHC can be performed on fixed formalin tissues. Most diagnostic laboratories have moved to IHC, especially with more available monospecific antiserums or monoclonal antibodies.

In recent years, antigen capture ELISA (ACE) assays have been developed and used both in state/university laboratories and private commercial laboratories. An example is the ACE assay for BVDV using fresh ear notches in PBS for detection of BVDV antigen. The ACE test detects the broad group of BVDV, but confirmation of BVDV types requires neutralization tests or genomic-specific tests such as polymerase chain reaction (PCR).

MOLECULAR DIAGNOSTICS FOR VIRAL GENOMIC MATERIAL

Currently with the known genomic sequences available, the detection of specific viruses can be made by polymerase reaction (PCR) for both RNA and DNA viruses. Initially the reverse transcription-PCR (RT-PCR) was commonly used for BVDV and other RNA viruses. As technology is available to the diagnostic laboratory, procedures such as real-time PCR are now commonplace for viral diagnosis. Viruses difficult to isolate in cell culture such as BRSV are now commonly identified by PCR. Rapid turnaround in hours versus days/weeks in cell culture is an advantage for PCR. The clinician should remain in contact with the diagnostic laboratory on the molecular diagnostic tests available; likewise, the diagnostic laboratory should explore this technology for its service.

SEROLOGIC TESTING

Diagnosis of an active infection with field strains of virus or response to vaccination with killed or modified live virus (MLV) strains can be made by detecting changes in antibody titers in acute to convalescent serum samples. An acute sample should be collected as early as possible in the course of infection/disease, and the convalescent sample 3 to 4 weeks later. It is appropriate to sample multiple animals from the same group, ideally with samples from both apparently healthy and diseased animals. A rise in antibody titer to the specific virus indicates exposure to that agent. A fourfold rise in antibody titer indicates an active infection when the microtiter virus neutralization (VNT) is used. The VNT in cell culture is routinely used for BHV-1, PI-3V, BRSV, and BVDV antibody testing. Recently some laboratories have incorporated ELISA tests for antibodies. The serologic testing for viral infections is both labor and time consuming. Plus time must evolve to get the acute and convalescent samples. Thus serology is retrospective at best and must be well planned to provide useful information. Often both diagnosticians and clinicians are frustrated when only one sample is available for the disease episode.

The prevention and control of viral infections of the respiratory tract focus on biosecurity and vaccination and, where possible, preventing exposure before immunity is established. Few, if any, antivirals are available for treatment of affected animals. When possible, vaccines are used in susceptible calves and selected vaccines are given to cows to boost transfer of immunity to the newborn.

BOVINE HERPESVIRUS-1

Bovine herpesvirus-1 (BHV-1) was first observed in the United States as an acute upper respiratory tract disease in cattle.[18] However, the first description of the disease was from Europe and was a vulvovaginitis in females.[19] Attention is often given to the change in management with large cattle populations in the changing feedlots, along with increased size of dairies resulting in cattle in close

proximity, facilitating spread of the virus. Manifestations of the BHV-1 clinical disease include respiratory tract disease, genital tract disease of the superficial surfaces, conjunctivitis, abortion, encephalitis, and generalized disease in the neonate. Researchers readily propagated the virus in cell culture, which led to development of vaccines relatively soon after the disease was characterized in the United States.[20,21] Because of potential losses for BHV-1–induced respiratory disease and abortions, BHV-1 vaccination programs are common in U.S. beef and dairy operations.

Etiology/Epidemiology

BHV-1 is a member of the viral family *herpesviridae,* subfamily *alphaherpesviridae*.[19] Three subtypes exist: BHV-1.1, BHV-1.2a, and BHV-1.2b. The BHV-1.1 are usually associated with respiratory and abortions, and BHV-1.2 is associated with genital tract infections.[19] A third subtype, BHV-1.2b, is not associated with abortion.[19] Experimentally the BHV-1.1 can cause genital infections. Likewise, BHV-1.2a can cause respiratory infections.[19] BHV-1.1, BHV-1.2a, and BHV-1 1.2b share antigenic properties but may be differentiated by restriction enzyme fragment polymorphisms (REFPs). A former term was *BHV-1.3,* which was from encephalitis cases. BHV-1.3 shares antigens with BHV-1.1 and BHV-1.2. Differences in the REFP enzyme profile exist between BHV-1.3 and the other subtypes, so this virus is now referred to as *BHV-5.*

BHV-1 infections are present worldwide in domestic cattle populations, both in beef cattle and dairies.[1] Goats are also susceptible, and other susceptible ruminants include the wild deer family members, water buffalo, and wildebeest. The disease occurs usually after recent additions to a herd, and the virus is transmitted to susceptible cattle.[1] The BHV-1 disease usually occurs after the calves have lost their maternal immunity. The disease is most common in cattle over 6 months of age.[1] The BHV-1 also survives in cattle recovering from primary infections with latency as a hallmark of the BHV-1 ecology.[1,19] The latent virus may be found in trigeminal and sacral ganglia with recrudescence later by stress or administration of corticosteroids.[1,19] After recrudescence/reactivation, the virus may be detected in nasal secretions with potential spread to contacts. Interestingly there may be an effective primary immune response acquired after natural infection or vaccination to control reexcretion (shedding).[19] A secondary immune response boosted by reactivation may also inhibit reexcretion.[19] Animals with high antibody BHV-1 titers (neutralizing) before reactivation did not reexcrete virus after reactivation treatment.[22] Cattle receiving MLV BHV-1 vaccines may also have latent infections with the vaccinal strains.[1,19] The host immunity to BHV-1 has been extensively studied with both T cells (cell-mediated immunity [CMI]) and B cells (humoral/antibodies) important in recovery and prevention on reexposure.[1,19]

Clinical Forms

Respiratory

The respiratory form may range from mild to severe disease. Also, inapparent infections may occur with later potential manifestations as abortions.[1] The respiratory form often occurs after new additions to a herd. The disease can be severe with morbidity up to 100% and a case fatality rate approaching 10%.[1] The disease severity is often due to other infections including *M. haemolytica, P. multocida, H. somni,* and/or *Mycoplasma* spp. The high transmissibility is evident—infectious virus may exceed 10^7 plaque-forming units in the nasal sections at peak shedding.[23] The viral shedding peaks at 3 to 6 days postinfection with clearance by day 12 to 14 after infection.[24] A relatively low dose of 10^3 to 10^4 infectious viral particles may cause infection; thus the virus may spread rapidly among susceptible cattle exposed to the animal shedding virus in close proximity.[25]

Clinical signs include fever, rhinitis, conjunctivitis, inappetence, and labored breathing. In dairy cattle, milk production may drop. Severe hyperemia of the muzzle and external nares may be evident, hence the term "red nose."[1,6,7] Pustules and diphtheritic plaques may be observed in the nasal mucosal of the external nasal passages. The cattle may survive the acute infection and disease, but if the respiratory disease does not resolve in 5 to 10 days, it is likely that secondary invaders may be responsible for severe pneumonia. The resulting death is most likely due to the pneumonia caused by the bacterial invaders. On occasion there can be cases of BHV-1 respiratory disease in feedlot cattle several weeks after the initial processing. Often these cattle had received BHV-1 vaccines at feedlot entry and processing.

The diagnosis of BHV-1 specifically requires laboratory confirmation. The white necrotic plaques observed in the external nares are suggestive of BHV-1. Nasal turbinates and the trachea may have severe inflammation with an adherent necrotic exudate. Primary lung lesions are not a feature normally seen in BHV-1 diseased cattle. The diagnostic testing includes nasal swabs from sick cattle for viral isolation in cell culture. The BHV-1 is one of the most readily/easily isolated viruses in cell culture with distinctive cytopathology. The agent is confirmed by immunofluorescence, neutralization of infectivity, PCR, or ELISA. Lesion material submitted for histopathology, usually from the nasal turbinates and trachea, may have intranuclear inclusions in addition to inflammation and necrosis. The formalin tissues can be examined by IHC to detect the BHV-1 antigen.

Serology is of potential value in surviving cattle with the admonition for both acute and convalescent serums collected 3 to 4 weeks apart to detect rising antibody levels by the VNT.

Conjunctivitis

BHV-1 conjunctivitis can occur as the only organ system involved. Yet it can occur with the respiratory form.[7] The disease is sometimes referred to as "winter pinkeye" because early descriptions were obtained from dairy cattle in the winter months, not normally associated with the time of *Moraxella bovis* induced disease of summer and insect vector involvement. The main signs are conjunctivitis with bilateral hyperemia and discharge. Corneal opacities occur and are in the periphery near the corneal scleral junction.[26] This is in contrast to the central corneal opacities caused by *M. bovis*.[26] The diagnosis can be confirmed by viral isolation in cell culture using swabs from

the affected eyes. Although conjunctivitis is the primary clinical sign, abortions can occur 1 or 2 months later.

Abortions

Abortions are relatively common sequelae to inapparent infections, respiratory disease, or the conjunctival form of BHV-1. Although a BHV-1 viremia is not easily detected in circulating leukocyte/monocytes, BHV-1 can infect the fetus in susceptible cows/heifers. The BHV-1 likely does not cause a viremia detected by viral isolation of blood leukocytes, but the virus may be detected by PCR in blood leukocytes.[19] Abortions can occur up to 100 days after initial infection.[1,7] The fetus is susceptible at any gestational age, yet most abortions occur after the fifth month of pregnancy. Abortions are usually observed at 4 to 8 months' gestation.[19] Susceptible females given most parenteral MLV vaccines containing BHV-1 may abort. Clinicians and owners must be aware of label indications because not all MLV BHV-1 vaccines are safe for pregnant female use. Aborted fetuses are usually dead when aborted with blood-tinged pleural and peritoneal fluids. The placental membranes may have to be removed manually. Diagnosis of BHV-1 is difficult in autolyzed fetal material, but when fresh placenta and fetal tissues are available to both histopathology and viral identification, diagnostic attempts can be rewarding. Intranuclear inclusions and focal areas of necrosis in the liver and adrenals are seen in BHV-1 abortions. The virus may be isolated from the placenta, fetal liver, and adrenals. Recently the use of contemporary BHV-1 reagents such as monoclonal antibody and IHC has enhanced BHV-1 abortion diagnosis. Serology using acute serum at time of abortion and a convalescent sample 3 to 4 weeks later in the aborting cow is unrewarding because the initial infection of the dam may have occurred as much as 100 days earlier, and thus she may already have seroconverted. Testing for BHV-1 antibodies in aborted fetuses is not useful because the fetus dies rapidly after infection, precluding immune stimulation with any detectable antibodies.

Genital Tract Infections

The genital tract form occurs in bulls and heifers/cows with the BHV-1.2 subtype.[1] Typically the acute infectious pustular vulvovaginitis (IPV) in the susceptible female occurs within 1 to 3 days of breeding by an infected bull.[1] Vesicles, pustules, ulcers, and plaques are observed on the mucosal surfaces of the vagina and vulva. The animals recover from primary infections in 10 to 14 days. Transient infertility may occur with subsequent bacterial infections causing metritis. The disease in bulls is similar to the female with incubation of 3 days with pustules, vesicles, and plaques on the penile and preputial mucosa (infectious balanoposthitis [IBP]). The virus may be isolated by cell culture using lesion material or swabs of affected lesions. Semen from infected bulls in the artificial insemination industry may contain BHV-1. Thus some AI bull studs may not permit entry of seropositive bulls as a precaution. Clinicians should be aware of such restrictions because BHV-1 vaccinations would likewise induce antibodies to BHV-1, as do natural field BHV-1 strains. Effective control of IPV and IBP because of BHV-1 by use of BHV-1 vaccines is unclear as to the vaccine efficacy against these genital tract lesions.

Central Nervous System Disease

Both BHV-1.1 and BHV-5 are capable of causing CNS disease, primarily encephalitis.[19,26] A nonsuppurative meningoencephalitis, suggesting viral origin, is observed on histopathologic examination. Clinical signs vary but may include excitement, incoordination, circling, recumbency, coma, and eventually death. Including both BHV-1.1 and BHV-5 in the differential diagnosis with rabies without inclusions is important to distinguish BHV CNS disease from rabies, making viral-specific diagnosis imperative. BHV1.1 or BHV-5 CNS disease is most likely confirmed after rabies diagnosis is negative. Tests such as immunofluorescence or IHC usually do not differentiate BHV-1.1 and BHV-5. Either unique monoclonal antibodies or REPF enzyme differences are required.

Generalized Disease

Typical for *alphaherpesviridae* family members, BHV-1 can also cause generalized disease of neonate calves.[26] Affected calves are either exposed in utero or immediately postpartum. This fatal form is associated with fever, anorexia, respiratory distress, conjunctivitis, and diarrhea. This high-mortality disease is associated with lesions such as necrosis and ulcers of the digestive tract and possible other organs. Microscopic lesions of adrenal necrosis may be evident. It has been suggested that generalized disease among neonate calves may occur simultaneous to the concurrent abortion storms caused by BHV-1.

Prevention and Control

Treatment for any form of primary BHV-1–induced disease is limited by lack of approved antivirals. Use of antimicrobials to minimize bacterial invaders in BHV-1 respiratory diseases is relatively common. Genital tract disease is usually self-limiting, and after recovery, the females return to the breeding program. Affected bulls recovering from IBP are problematic for safe use in the natural breeding herd. The encephalitis and generalized forms are rare in occurrence and are largely dealt with in the differential diagnosis considerations of the respective organ system at necropsy.

Basic control of BHV-1 deals with biosecurity and vaccination. Where possible, cattle with signs of overt BHV-1 should not enter the herd; however, isolation on entry for 30 to 45 days would be compatible with other programs such as Johne's disease or BVDV prevention. Some countries and AI bull studs have moved to monitoring for BHV-1 by serology. Then the seropositive BHV-1 animals may be denied movement into these geographic regions or AI facilities. It is assumed that seropositive status indicates BHV-1 infection such as latency in CNS tissues. Potentially seropositive animals, when stressed or given corticosteroids, may undergo viral recrudescence resulting in viral shedding. However, using serology alone has its drawbacks for detection of potentially infected animals. Many animals have only a low BHV-1 antibody titer or no detectable antibody titer after recovery from field infections or vaccination. Thus relying solely on

antibody testing may not be successful to detect latent infections.

In cattle operations and geographic regions (countries) with an already herd level of infection, the approach to controlling the disease is via vaccination.

More than 165 vaccines against BHV-1 are available in the United States for use in cattle.[27] Vaccination protocols for beef and dairy cattle in the United States routinely incorporate use of one or more vaccines against BHV-1. These vaccines are classified into five types: (1) MLV vaccines for parenteral administration (intramuscular and/or subcutaneous); (2) MLV, intranasally administered vaccines; (3) chemically altered, live virus, temperature-sensitive vaccine for parenteral use; (4) inactivated viral vaccines for parenteral use; and (5) a combination of parenteral BHV-1 MLV and inactivated BHV-1 viral vaccine. These vaccines may be single-component (monovalent) vaccines (e.g., BHV-1 alone) or may contain several immunogens including various combinations of BVDV types 1 and 2; PI-3V; BRSV*, Leptospira* spp., *serovars; H. somni; M. haemolytica; P. multocida;* and/or *Campylobacter* spp.

BHV-1 MLV parenteral vaccines induce both B-cell (humoral) and T-cell (cell-mediated) active immune responses after one dose of MLV vaccine.[28] Serum antibodies to BHV-1 along with BHV-1 specific CD4+, CD8+, and γδ T cells were detected after BHV-1 MLV vaccination.[28] Calves born to dams with circulating BHV-1 antibodies may absorb colostrum-derived maternal antibodies to BHV-1 and other viruses.[16] The mean half-life of viral antibodies to BHV-1 in calves receiving maternal immunity was 21.2 days.[16] Potentially, calves receiving passive immunity to BHV-1 may have reduced response to BHV-1.[29] Calves seronegative to BHV-1 were given BHV-1 neutralizing antibody intramuscularly and subsequently given MLV BHV-1 intranasally. The passive BHV-1 immunity via BHV Ig had a reduction on the efficacy of the MLV BHV-1.[29] The passively administered BHV-1 antibodies protected against viral shedding in viral-challenged calves.[29]

The MLV parenteral vaccines were the initially licensed for use in cattle for protection against BHV-1.[30] Vaccines are attenuated by multiple passages in cell culture and/or in heterologous species' cell cultures and often retain their ability to replicate in a susceptible animal, possibly causing a viremia. MLV parenteral vaccines are relatively inexpensive, offer a convenient route of administration, and stimulate a rapid onset of immunity (i.e., within 3 days of administration).[31-33] In general, one dose given to a susceptible animal stimulates protective immunity, which varies in duration depending on the clinical form of the disease challenge. Calves receiving a combination MLV vaccine including BHV-1 were protected for at least 126 days after vaccination as measured by protection against infection.[34] The MLV parenteral vaccines may cross the placenta and infect the fetus, causing abortion.[35] Most MLV BHV-1 parenteral vaccines are not approved for use in pregnant heifers/cows or nursing calves.[27] Recently companies have received label claims for BHV-1 and BVDV MLV vaccine use in pregnant cows providing they vaccinated with that line of vaccines within 12 months and to nursing calves provided their dams were vaccinated within 12 months.[27]

MLV intranasal vaccines generally can be divided into two types, based on the attenuation process: (1) those modified by passage in a cell culture[36,37] and (2) those modified by treatment such that they become "temperature sensitive"[38] (i.e., they do not replicate at internal body temperature). MLV intranasal vaccines stimulate protection in susceptible animals with only one dose, in contrast to the chemically altered MLV parenteral vaccines. The label directions for selected, but not all, MLV intranasal vaccines may indicate that they can be safely used in pregnant cattle.[27] These vaccines induce a rapid onset of protection (within 3 days of administration), possibly through interferon production and release into nasal secretions.[36] One benefit of MLV intranasal vaccines is that they stimulate immunity to mucosal surfaces of the upper respiratory tract, the portal of entry of the virus. Another benefit is their potential to immunize calves that are already seropositive because of maternal (humoral) antibodies passively transferred through the colostrum.[39] Animals vaccinated with the MLV intranasal vaccines may transiently shed virus in the nasal secretions and therefore might infect susceptible contact animals.[40]

The chemically altered BHV-1 vaccine strain for parenteral use was modified by nitrous acid treatment, which caused changes in the viral genome resulting in a strain (temperature sensitive) that is unable to replicate at normal internal body temperature.[41] Presumably, because of the limited viral replication, the vaccine requires two doses to stimulate immunity. Because it is temperature sensitive and should not replicate in the host, the vaccine can be used in pregnant cattle.[27,41,42] In one study heifers received two doses of the vaccine and were challenged with BHV-1 7 months later (at 6 months' gestation). These heifers showed a significant reduction in the number of abortions and stillbirths compared with controls.[42]

Inactivated viral vaccines are prepared by growing virus in cell cultures and then inactivating them with chemicals. An adjuvant is added to the inactivated strain to help stimulate an immune response. Inactivated BHV-1 vaccines require two doses (14-28 days apart) when used for the initial vaccination of susceptible cattle. Historically it has been thought that inactivated vaccines against viruses did not induce as long a duration of immunity as the MLV vaccines, nor did they confer protection against mucosal infections. Controlled studies are required to determine the duration of immunity induced by inactivated BHV-1 vaccines and MLV vaccines, both for respiratory disease and fetal infections. Disadvantages of inactivated vaccines are that the onset of protection may not be as rapid as with MLV parenteral or MLV intranasal vaccines and two doses are required. An advantage of the inactivated vaccines is that they can be used in pregnant cows and nursing calves.

Many vaccines are available for preventing and controlling the different forms of BHV-1 disease, and each vaccine has certain characteristics that should be considered when designing vaccination programs for various types of cattle operations and managements. Each vaccine also has both benefits and limitations. Probably more important is the management of the cattle for which the vaccines are used.

The MLV parenteral vaccines may infect the fetus if pregnant susceptible heifers or cows are vaccinated. Abortions have been reported subsequent to vaccination with MLV parenteral vaccines.[35] The MLV vaccine virus may also result in corpus luteum infection or disease. Experimental studies have indicated a reduced conception rate in susceptible cattle that received an MLV parenteral vaccine 3 to 4 days before or 14 days after breeding.[43,44] Clinical observations indicate that susceptible recipients used in embryo transfer (ET) may have delayed estrous after MLV parenteral (BHV-1 and BVDV) vaccine use and synchronization. It has been reported that pregnant cattle raised in contact with calves recently vaccinated with MLV parenteral vaccines had a greater incidence of BHV-1 abortion than those that did not have contact with vaccinates.[45] Consequently, the labels of MLV parenteral vaccines have usually stated that the vaccine should not be used in calves nursing pregnant cows. Recent studies, however, have shown that calves given an MLV parenteral vaccine did not shed virus in their nasal secretions nor did contact animals become infected with the vaccine virus.[46-48] Multiple companies have received label claims for MLV vaccines containing BHV-1 and BVDV for pregnant cows provided the cows had received the same line of vaccines with the MLV BHV-1 and BVDV within 12 months and/or before breeding. Likewise, these vaccines could be used in nursing calves if cows previously vaccinated with that line of vaccines according to the label. Veterinarians and producers should follow explicitly the label precautions for the respective vaccine. Another concern is that the MLV vaccine virus may recrudesce, with resulting shedding of virus in cattle either stressed or receiving corticosteroids.[49] Realistically, concern about transmission of BHV-1 to animals in contact with those receiving MLV parenteral vaccines would be negligible if the contact animals were properly immunized and immune to BHV-1.

Until the vaccine labels on most MLV parenteral vaccines are changed, MLV intranasal vaccines or the inactivated or chemically altered live virus vaccines are usually recommended for pregnant cattle or those near breeding. The exceptions are the approved vaccines for use in pregnant cattle and nursing calves. Vaccine recommendations should be weighed, with the benefits of vaccination as a guide and especially with the realization that properly vaccinated cattle are better protected when exposed to either field (virulent) or vaccine strains shed by vaccinated animals.

Cattle that are susceptible and likely to be exposed to BHV-1 should receive either an MLV parenteral vaccine or an MLV intranasal vaccine because both types induce immunity within 3 days of the initial dose. Rapid onset of immunity is desirable in such situations as stocker calf and feedlot operations, in which calves are transported long distances to pastures or feedlots, which stresses the animals and makes them more susceptible to infection. Such calves are also exposed to infection with BHV-1 from contact cattle in the markets. The drawback to inactivated vaccines is that two doses are required to obtain good immunity.

Controlled studies on the duration of immunity are limited. A degree of protection against challenge existed at 6 to 9 months after vaccination with an MLV intranasal vaccine or an inactivated vaccine.[50,51] A parenteral MLV BHV-1 MLV vaccine provided protection up to 126 days after vaccination.[34] Challenge studies for licensure are usually performed on calves within days of vaccination, at the time of peak immunity. Also, the challenge may be for only one form of disease, usually the respiratory type. Such challenges may detect only protection against a severe form of the respiratory disease. BHV-1 manifests itself in other forms such as abortions, neonatal disease, genital disease (male and female), and conjunctivitis. Yet little or no data are available about the efficacy of vaccines against these other forms of disease. For example, in one case the genital form of BHV-1 disease (infectious pustular vulvovaginitis) occurred in heifers that had received an MLV parenteral vaccine 5 months earlier.[52] Given the lack of duration of immunity studies for all BHV-1 vaccines individually and the cost of vaccines, breeding animals are usually vaccinated at least annually. In some feedyard situations the animals may be revaccinated during the feeding period. It is industry practice that feedlot cattle receive a monovalent BHV-1 MLV parenteral vaccine at reimplant time at approximately 100 days after arrival. There have been field reports of BHV-1 respiratory disease (IBR) in feedlot cattle after a few months of entry/processing, at which time they received MLV vaccines containing BHV-1.

The possibility exists that maternal BHV-1 antibodies acquired by the calf through ingestion and absorption of colostrum may interfere with vaccination. The level of these serum BHV-1 antibodies in the calf depend on the amount in the colostrum, amount absorbed, and half-life of the particular antibody; for BHV-1, 21.2 days.[16] Some calves receive no BHV-1 antibodies through the colostrum, or they may lose them within 1 month. Some calves, however, may have serum BHV-1 antibodies for up to 6 months after birth.[49]

Vaccination recommendations for neonatal calves include use of multiple doses of an MLV parenteral, an inactivated, or a chemically altered live virus vaccine or administration of an MLV intranasal vaccine. The maternal antibodies may block the parenterally administered MLV or inactivated vaccine. However, the MLV intranasal vaccine may still induce BHV-1 immunity.[39] Calves are often revaccinated at 6 to 8 months of age regardless of their prior vaccination history.

Molecular techniques of biotechnology have been applied to the study of vaccines and the response to vaccination (vaccinology). These advances are especially noted for herpesviruses including BHV-1. In addition to conventional vaccines manufactured via propagation of MLV and inactivated BHV-1 strains, current and future technologies offer opportunities for other vaccines.[53,54] These include subunit vaccines with a portion of the virus, deletion mutants with specific viral genomic fragments deleted, live vectored strains, DNA vaccines using plasmids, and plant-based vaccines. Deletion mutant BHV-1 vaccines as marker vaccines with selected glycoprotein genes deleted along with diagnostic tests for the deleted genes permit identification of vaccinates under control programs.[53] Recently needle-free delivery of vaccines has been developed and implemented.[53] By high-pressure gas delivery, vaccines may penetrate the skin and

be administered intradermally, subcutaneously, or intramuscularly.[53] Such delivery is designed to minimize damage resulting from intramuscular injections. Two studies compared needle-free intramuscular injection of multivalent MLV vaccine containing BHV-1 with conventional subcutaneous injection via syringe in dairy calves and feedlot cattle. In both studies antibody titers to BHV-1 were higher at day 21 postvaccination than conventional needle injection.[55,56]

The best possible vaccine provides protective immunity in the host against infection (viral replication) when challenged, protects the animal against all forms of disease including multiple organ and systemic forms, and provides lifelong mucosal and systemic immunity. Ideally the vaccine recommendations would incorporate the results of field trials that are carefully designed to show the efficacy of the vaccine against a pathogen. Unfortunately little information is available, as can be seen by a review of the literature, for evaluating the field efficacy of the respiratory disease vaccines.[57] The summary of results was mixed for BHV-1 vaccines and for other respiratory viral and bacterial vaccines.

Calves may be vaccinated at weaning or 30 days before weaning. Calves vaccinated before 6 months of age should be revaccinated because the earlier vaccination may have been blocked by maternal antibodies. The MLV parenteral and intranasal vaccines require only one dose in susceptible calves, whereas the chemically altered live virus or inactivated vaccines require two doses. Although the labels for most MLV parenteral vaccines state that the vaccine should not be used if the calf is nursing a pregnant cow, the likelihood of infection of the pregnant cow may be minimal, especially if she is already immune. Yet as described earlier, MLV parenteral vaccines are available for use in pregnant cows and nursing calves.

Yearling heifers (12-14 months of age) should be vaccinated at least 1 month before breeding. Any of the vaccines may be used, but if two doses are required, the second dose should be given at least 1 month before breeding.

Pregnant cows may be vaccinated with a vaccine that has a label description permitting such use; these include MLV intranasal vaccines, chemically altered live virus vaccines, inactivated vaccines, and approved MLV parenteral vaccines. Generally one dose is used, primarily because of management considerations. Administering booster doses of the BHV-1 vaccines may have two conflicting outcomes as a result of booster dose stimulation of an increase in colostral BHV-1 antibodies, which are transferred to the newborn calf in the colostrum; consequently, (1) it may be beneficial to the calf to have increased BHV-1 serum antibodies for protection against BHV-1 disease, or (2) the calf may have longer duration of BHV-1 antibodies, which may block BHV-1 immunization. No multiyear-duration-of-immunity studies in vaccinated cattle challenged with virulent BHV-1 have been published. Because of the relatively low cost of BHV-1 vaccines and the need to vaccinate against other pathogens, many breeding cows are given BHV-1 vaccine annually.

Cattle to be shipped to forage pasture after weaning (wheat pasture or native grass) or to feedyards should be vaccinated 2 to 3 weeks before shipment. However, management practices and marketing may only permit vaccination at the initial collection point, market site, or stocker/feedlot delivery. All the major types of BHV-1 vaccines may be used, but those that require only one dose have two advantages: rapid onset of immunity and less handling required (one dose vs. two).

Cattle presented for purchase immediately before shipment, with no known vaccination history, pose a challenge. Presumably healthy cattle may be candidates for the one-dose MLV parenteral or MLV intranasal vaccines because these calves may benefit from rapid immunity. Cattle already infected with BHV-1 may not be protected by vaccination.

Cattle entering the feedyard usually receive either the MLV parenteral or MLV intranasal vaccine, particularly for the rapid onset of immunity. Cattle in the feedlot are routinely revaccinated later during the feeding period (at reimplant time) to ensure protection against possible BHV-1 disease occurring several weeks late in the feeding period.

Veterinarians should consult the breeding bull center for vaccination requirements of bulls, especially relating to export shipment and collection for artificial insemination (AI). Potentially the MLV BHV-1 vaccines including intranasal vaccines could induce latent infections and also stimulate antibody production.[58] Surveillance for BHV-1 includes serotesting, and potentially antibody-positive bulls could be disqualified for AI purposes.

BOVINE RESPIRATORY SYNCYTIAL VIRUS

Bovine respiratory syncytial virus (BRSV) is one of several viruses causing respiratory tract infection and disease in cattle. The BRSV infections range from inapparent and mild to severe respiratory tract disease.[2] The BRSV can be a single etiologic agent; participate with other viruses; and/or damage the respiratory tract disease, allowing secondary invaders entry and environment for more severe BRD with pneumonia. BRSV appears limited to the respiratory tract with no effects on reproduction and/or fetal disease.

Etiology/Epidemiology

The BRSV is a member of the genus *Pneumovirus* of the family Paramyxoviridae.[2] The virus replicates in cell culture, permitting cell culture propagation for serology, viral isolation, and vaccine production. Because this is an enveloped virus, it is susceptible to the environment and disinfectants. Sheep and goats are susceptible to BRSV but are not likely important as a reservoir for exposing cattle. An RSV for goats exists. Although there are possible antigenic differences among BRSV strains, they are believed to have one major antigenic type. Cattle are the reservoirs of infection serving as the source of exposure to susceptible cattle.[2,7] Numerous serosurveys indicate that BRSV antibody-positive cattle had not received BRSV vaccinations. In general the disease occurs in the younger cattle, 3- to 12-month-old calves. Aged/adult cattle with BRSV disease, either in feedlots or dairy cows, have been reported.[2,7] Spread of the virus is via infected respiratory tract secretions, and the virus can move quite rapidly in a susceptible population.

Clinical Disease

Infections with BRSV may be inapparent, cause primary respiratory tract disease, or cause damage to the respiratory tract with bacteria such as *M. haemolytica, P. multocida, H. somni,* and *Mycoplasma* spp.[2,7,8,12,59] Often in epizootics of BRD there will be seroconversions in both healthy and diseased cattle with no difference in seroconversion rates of both groups.[8,12,59] The BRSV infects epithelial cells from the nasal mucosa to the bronchi including the type II pneumocytes and alveolar macrophages.[2] Loss of cilia and necrosis of bronchial and bronchiolar epithelial cells occurs with BRSV infection. Similar to human RSV in infants, a hypersensitivity causing bovine respiratory disease has been suggested. Because of widespread use of killed and MLV vaccines, this hypothesis for severe disease was advanced. However, despite numerous experiments, this has not been conclusively determined to be a hypersensitivity affecting the bovine respiratory tract that results in clinical disease in cattle.

The clinical signs are limited to those of respiratory disease with fever, coughing, nasal discharge, and ocular discharges.[2,7] Severely affected cattle may have severe respiratory distress. Mouth breathing along with subcutaneous emphysema is observed on occasion. In some instances BRSV disease has been observed in late feeding periods in the feedlots. Attempts were made to implicate BRSV as a severe respiratory disease problem occurring late in the feedlot, atypical interstitial pneumonia (AIP). So far a clear connection of BRSV and AIP has not been established. Necropsy lesions in affected BRSV cases reveal a diffuse interstitial pneumonia with subpleural and interstitial edema.[2,7] Pulmonary emphysema may be present as well. Microscopic lesions may reveal multinucleated (syncytia) in the bronchiolar epithelium and lung parenchyma. Intracytoplasmic inclusions may also be present.[2,7] Often the bacterial secondary invaders may cause severe pathology resulting in bronchopneumonia or fibrinous pneumonia.

Diagnosis

The lesions, although suggestive of those caused by *paramyxovirus* family viruses, are not by themselves diagnostic for BRSV. The virus must be identified by viral isolation in cell culture from nasal swabs or lesion materials. However, the virus is quite labile and rarely isolated in cell culture. PCR tests in infected nasal swabs can detect BRSV.[60] However, better reagents assist use of immunofluorescence in tissues. More recently, IHC testing of lung tissues has increased the diagnostic capability for BRSV. Some laboratories have used human RSV ELISA kits to detect BRSV antigens. Serology using acute and convalescent serums may detect active infections as supported by a fourfold rise in titers in neutralization tests. Clinicians submitting samples to diagnostic laboratories should inquire beforehand about samples to be collected and shipping conditions.

Prevention and Control

Antibiotics to lessen effects of bacterial infections, and sometimes antihistamines, have been used for treatment.

The prevention of BRSV relies heavily on use of MLV and killed BRSV vaccines, and there are numerous vaccines in the United States, usually in combination with BHV-1, PI-3V, BVDV, and bacterial immunogens.[27] These multicomponent vaccines with BHV-1, PI-3V, BVDV, and BRSV are standard for vaccination programs in both beef and dairy operations. These BRSV vaccines are parenterally administered vaccines. No licensed intranasal BRSV vaccines are available in the United States. Most initial vaccine regimens use two doses, 1 to 4 weeks apart. Annual revaccinations are included in both beef and dairy operations. BRSV protection for both beef stocker operations and feedlot entry by routine vaccination is standard industry practice.

BOVINE PARAINFLUENZA-3 VIRUS

Bovine parainfluenza-3 virus (PI3V) is a relatively common infection in domestic cattle.[4,6,7] Similar to BRSV, BVDV, and other viruses, the PI-3 infections are both inapparent and sometimes associated with clinical signs and mortality with respiratory tract disease. The PI-3V should be considered both as a primary invader, but likely more importantly as a virus capable of compromising the bovine respiratory tract for secondary invaders. The PI-3V is limited to the respiratory tract causing no other diseases for the digestive tract, CNS, or fetal infections. The PI-3 virus immunogen is included in almost all killed and MLV bovine vaccines.

Etiology/Epidemiology

PI-3V is a member of the *Paramyxovirus* genus of the viral family Paramyxoviridae.[4,7] This RNA virus also contains an envelope and is thus susceptible to the environment and disinfectants. Cattle are the major host, although sheep, goats, and wild ruminants are susceptible.[4,6] This virus followed BHV-1 in its initial isolation in cell culture and characterization from cattle with BRD. This *paramyxovirus* is readily propagated in cell cultures with cytopathology, and it also causes hemagglutination with RBC. Virus neutralization tests and hemagglutination inhibition tests are used for serology.

Clinical Disease

The PI-3V is limited to respiratory tract infections with epithelial cells from the trachea bronchi and alveoli affected.[4,6,7] The ciliated epithelial cells are necrosed with resulting altered mucociliary clearance. Clinical signs include fever, coughing, nasal and ocular discharges, and altered lung sounds suggesting pneumonia. An incubation of between 24 and 36 hours with a subsequent fever with the previously mentioned clinical signs in a primary PI-3V infection with the calf usually recovering is the norm. However, as with other bovine viruses altering the respiratory tract, secondary invaders complicate the disease with often severe bacterial pneumonia. Interestingly, it is not unusual for healthy calves to seroconvert without BRD signs after arrival in facilities directly from the ranch. PI-3V infections often occur in both healthy and diseased cattle (unvaccinated) commingled for

30 to 35 days after sale barn acquisition and entering the feedlot.[8,9,12]

Diagnosis

Lesions in primary PI-3V respiratory disease are minimal with mild pneumonic lesions, interstitial pneumonia, and intracytoplasmic inclusion in various regions of the nasal mucosa to developing syncytia in the lung.[4,6,7]

The virus may be isolated in cell cultures from nasal swabs and lung tissues at necropsy. Immunofluorescence and IHC are also available to detect the viral antigen in affected tissues. VNT tests and hemagglutination inhibition antibody tests are available to detect rising antibody levels in acute and convalescent serums.

Prevention and Control

Prevention involves both killed and MLV vaccines using parenteral administered vaccines.[27] A limited number of MLV vaccines are given intranasally.[27] Experts have mixed attitudes about the pathogenic potential of PI-3V, with some referring to PI-3V as limited to inapparent infections or minimal signs or lesions. Yet PI-3V has been isolated from sick cattle and severe pneumonias at necropsy, albeit with secondary bacteria. The PI-3V vaccine immunogens were readily incorporated into the BHV-1 vaccines and subsequently with BVDV in the 1960s and remain there today along with BRSV. No adverse effects of PI-3 immunogens are apparent, and some proponents feel that the PI-3V vaccines may be beneficial.

BOVINE ADENOVIRUSES

Bovine adenoviral infections are likely evident in cattle populations worldwide.[13] Bovine adenoviruses (BAVs) have been found in both the respiratory and/or digestive tract in either inapparently infected or diseased cattle.

Etiology/Epidemiology

BAVs are members of DNA viral family Adenoviridae.[13] Ten recognized serotypes of bovine adenoviruses are available.[61] These are divided into two genera, *Mastadenovirus* and *Atadenovirus*. *Mastadenovirus* genus contains BAV1, 2, 3, 9, and 10 serotypes and *Atadenovirus* 4, 5, 6, 7, and 8 serotypes. These serotypes are based on viral neutralization tests. The Adenoviridae family is well known for infections in affected organs with intranuclear inclusion bodies. Based on serosurveys for viral antibodies, the BAV are quite common in cattle. As with BRSV and PI-3V, it is not unusual for calves held after shipment and commingled to seroconvert with no apparent disease and BAVs may be found in cattle with other viruses such as BVDV.[14]

Clinical Infections

Experts believe that with BAVs causing respiratory and digestive tract infections, respiratory secretions and feces could have infectious virus for transmission.[1] BAVs have been found in inapparently infected healthy calves, as well as in selected cattle with respiratory disease or digestive tract disease.[13] Reports on field studies trying to establish disease potential for BAV are mixed. Experimental studies with BAV challenges have resulted in no lesions or limited respiratory or digestive tract disease lesions.[13]

Diagnosis

The diagnostic laboratory may find an occasional cell culture isolate with viral cytopathology in affected cells with both samples from healthy or diseased animals at necropsy, or possibly from nasal swabs or fecal samples. The lack of envelope on the agent showing resistance to ether or chloroform as lipid solvents points to adenoviruses, which lack an envelope. Sometimes antisera to BAV are available to confirm the virus, or electron microscopy may detect morphology of adenoviruses.

Prevention and Control

No licensed or marketed BAV vaccines are available in North America, nor does there appear to be justification to develop the BAV vaccines.

BOVINE CORONAVIRUS

Bovine coronaviruses (BCVs) were initially associated with neonatal calf diarrhea.[15] Then BCVs were identified with "winter dysentery" in adult dairy cattle.[7,15] Later BCVs were detected in respiratory secretions of infected calves with subsequent isolation from cattle with BRD signs. This isolation of BCVs from calves with "shipping fever" pneumonias led to the assumption that BCVs were a major etiology for BRD. In some studies other agents such as BRSV, BVDV, and PI-3V along with bacteria were also found in these severely ill cattle. No doubt BCVs are found in conjunction with other respiratory tract infections, yet their sole or primary BRD role has not been clearly established. Including BCV along with other bovine respiratory tract viruses contributing to BRD is best. Clearly, experimental reproduction of detectable and severe respiratory tract disease such as pneumonia would better make the case for BCV as a significant primary pathogen in respiratory tract disease in cattle.

Etiology/Epidemiology

BCVs are RNA viruses of the viral family Coronaviridae.[6,15] They are enveloped viruses, thus sensitive to disinfectants and the environment. It is not unexpected that cattle would have a coronavirus with tropism for the respiratory tract. Coronaviruses infect the respiratory tract of other species including humans, pigs, turkeys, and chickens. BCV infections in cattle are worldwide. Initially implicated in neonatal calf diarrhea, BCVs were also reported with etiology in "winter dysentery" of adult cattle. Subsequently BCVs have been isolated from the nasal samples of cattle undergoing respiratory tract disease.[62-66] Thus this virus has a purported role in both respiratory tract disease and enteric diseases. Only one serotype is recognized, but likely there is some antigenic variability.[15] The dilemma for working with BCV experimentally and

diagnostic laboratories attempting to isolate the virus is that BCV replicates poorly or is quite difficult to isolate in standard cell cultures. A specialized cell line, a human rectal adenocarcinoma line, is permissive for BCV and has been used for virus isolation from feces and nasal swabs by selected laboratories.

The BCV is considered relatively common in enteric infections in both beef and dairy operations. The virus has been isolated from cells with disease including calf pneumonias, as well as beef cattle entering feedlots in various U.S. regions. The BCV was isolated from both healthy and sick cattle in these BRD episodes. And BCV was detected by seroconversions during the first month in feedlots in transported cattle.

Clinical Disease

The association of BCV with BRD has been primarily by the isolation of virus from nasal swabs of cattle with BRD signs and seroconversions to BCV. The virus has been found in healthy calves as well. Likewise, antibody testing has detected seroconversions in cattle in BRD cases. The clinical signs in the BRD cases are not unlike other BRD cases with viral etiologies present such as BVDV; PI-3V; BRSV; and other viruses with fever, nasal and ocular discharges, anorexia, and coughing. Typically these BCV isolations and seroconversions occur soon after arrival to the feedlot. As expected there is often involvement of secondary bacteria such as *M. haemolytica* and/or *P. multocida*.

Attempts have been made to demonstrate the pathogenicity of BCV for the bovine respiratory tract. After experimental challenge in young calves, the virus could be found in feces of diarrheic calves and nasal swabs for up to 5 days.[15] Respiratory disease signs occurred in only a few calves. Lesions of emphysema and interstitial pneumonia were evident in only a few calves.[67,68] For other studies, there are mixed reports of BCV detected in lung tissues of cattle with BRD, one report with no BCV detection in lungs of cattle with BRD,[6] and another detecting BCV antigen by immunofluorescence in respiratory tissues.[69]

Diagnosis

The virus can be isolated in cell culture provided that a unique cell culture is available to the diagnostic laboratory, the human rectal adenocarcinoma line (HRT-18).[15] The nasal swabs collected appear to be the choice of collections from live cattle for testing. An antigen capture ELISA originally used for detecting BCV antigen in fecal samples is also used by some diagnostic laboratories for BCV detection in respiratory disease samples. Also, BCV immunofluorescence is available to detect BCV antigen. Selected diagnostic laboratories and research units have used PCR to detect BCV in diagnostic samples. Use of electron microscopy could detect BCV in respiratory samples similar to the use of EM for fecal samples. Selected research laboratories have used ELISA tests for BCV antibodies, and in some selected studies they found seroconversions when paired samples were available. Clinicians should consult with their respective diagnostic laboratory for their testing for BCV.

Prevention and Control

Although there are licensed BCV vaccines for enteric disease protection, there are no licensed BCV vaccines in the United States to control respiratory tract disease in cattle. Treatment focuses on the use of antimicrobials to control the bacterial secondary infections. As in prevention of the neonatal enteric disease, it is assumed that adequate colostrum is available to provide protection in the young calf.

BOVINE VIRAL DIARRHEA VIRUS

Bovine viral diarrhea viruses (BVDVs) are a diverse group of viruses that cause infections in domestic ruminants worldwide. The virus is responsible for considerable economic losses from morbidity, mortality, loss of production (milk), reproduction losses, reduced feedlot performance, cost of treatments and prophylaxis, and cost of control measures. The infections range from inapparent to severe, with pathology involving single or multiple organ systems.

Two major aspects of BVDVs have brought the group of BVDVs to the forefront: (1) the presence of persistently infected (PI) cattle as the reservoir of infection; and (2) availability of tests such as the immunohistochemistry (IHC) and antigen capture ELISA (ACE) to detect PI cattle. These diagnostic tests permit more effective and timely identification and removal of PI cattle, minimizing virus exposure in susceptible cattle.

BVDVs often interact with other infectious agents contributing to substantial respiratory and digestive tract diseases. Together with fetal infections, these manifestations of disease have focused continual scrutiny for BVDVs in several forms.

Numerous detailed reviews/books of BVDV are available with considerable detailed information on BVDV. These references should benefit all clinicians, diagnosticians, researchers, and producers interested in BVDV.[3,70-73] For extensive and specific citations and references to the BVDV following coverage, readers should consult these extensive reviews/books.[3,70-73]

Etiology/Epidemiology

BVDVs are members of the Flaviviridae family, *Pestivirus* genus along with classical swine fever/hog cholera virus and border disease virus of sheep.[74-76] The BVDV is a single-stranded RNA of 12.5-kb length, translated into a polyprotein.[74-76] The protein is cleaved into individual proteins by cellular and viral proteases.

The four viral structural proteins are the capsid protein (C) and three glycoproteins of the envelope (Erns [ribonuclease], E1, and E2) at the 5′ region of the viral genome (Fig. 42-1). These three glycoproteins (GP) are involved with the induction of neutralizing antibodies. The E2 (GP53) glycoprotein is considered the principal epitope for viral neutralizing antibodies. The nonstructural proteins (NS) are toward the 3′ region of genome. The biotypes of BVDV are based on presence or absence of cytopathology in cell cultures, cytopathic (CP) or noncytopathic (NCP).[3,70-73] The NCP strains encode for an

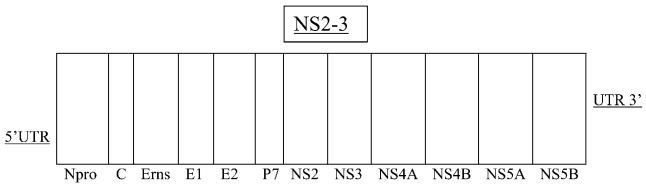

Fig 42-1 Bovine viral diarrhea virus genome and proteins encoded.

intact NS2-3 protein; however, the CP strains have the NS2-3 cleaved into separate NS2 and NS3 proteins. This is a molecular event separating the CP and NCP biotypes. Although not part of the assembled virion, the individual NS proteins may have functions including protease and polymerase activity. Genomic variabilities encode for these proteins, and there is diversity noted by monoclonal binding studies.[77]

The BVDVs are a diverse group, both by antigenic and genomic properties.[78-84]

Until recently in the United States, BVDVs were divided into two major genotypes, BVDV1 and BVDV2. In the United States there are three major subtypes: BVDV1a, 1b, and 2a. One isolate of BVDV2b in the United States has been reported from a feedlot case of fatal pneumonia.[11] Reports indicate that there are 11 subgenotypes of BVDV1.[85] The phylogenetic analysis of BVDV can be performed by analyzing genomic sequences of five different regions: 5'-UTR, Npro, E2, NS3, and NS5B-3'UTR.[86] Based on homology using a 5'-UTR region, there was 67.1% homology between BVDV1 (including BVDV1a and BVDV1b) and BVDV2a strains.[81] And there was 85.3% homology between BVDV1a and BVDV1b strains.[81]

BVDVs cause important and commonplace infections in domestic cattle worldwide.[3] In addition, other susceptible domestic species include sheep (also susceptible to the related border disease virus), goats, pigs, and a wide range of wild ruminants, as well as camelidae and cervidae species.[3] The most important reservoirs of the virus are the PI cattle. PI cattle are the result of susceptible pregnant cattle becoming infected between 42 and 125 days of gestation.[87] The infected fetuses are carried to term, born infected and immunotolerant to the BVDV strain causing the infection. The calves are lifelong shedders of the virus in all body excretions/secretions including feces. Animals with acute, transient infections shed virus at far smaller amounts and for a shorter time than PI cattle.[3]

The distribution of the BVDV subtypes in the United States has been reported in two groups of livestock: BVDV isolates from cases submitted to diagnostic laboratories and from PI cattle in various management schemes. The prevalence of BVDV biotypes and subtypes was determined in BVDV-positive samples from diagnostic laboratory accessions.[88] The results were 89.3% NCP strains, 8.4% CP strains, and 2.3% in which both CP and NCP were isolated. The distribution of the BVDV subtypes was 45.8% BVDV1b, 28.2% BVDV1a, and 26% BVDV2a. In a survey of Northwestern U.S. diagnostic laboratory accessions, there were 18.5% BVDV1a, 40.7% BVDV1b, and 40.7% BVDV2.[89] A survey of BVDV-positive samples from bulk milk and infected cattle indicated 49.1% BVDV1b, 11.3% BVDV1a, and 39.3% BVDV2a. [90] Thus it appears that in the United States, BVDV1b is the predominant subtype in diagnostic laboratory cases.

The prevalence of PI cattle in the United States was reported in two surveys.[91-93] In a multistate study of 18,931 cattle, there was a PI positive rate of 0.17%.[91] Studies of prevalence of PI cattle entering feedlots indicated 0.3% and 0.4% BVDV PI.[92,93] The PI strains from a feedlot study indicated that BVDV1b was the predominant subtype; BVDV1b, 77.9%; BVDV1a, 11.6%; and BVDV2a, 10.5%.[93]

Transmission of BVDV is by direct or close contact with infected cattle (horizontal transmission).[3] Direct contact with infectious secretions or aerosols from PI cattle are the most likely sources of infection. In PI animals, BVDV is present in the serous secretory and ductular epithelium from the nasal mucosa to the lung, along with infected respiratory tract leukocytes.[94] The potential for iatrogenic mechanical transmission via infected veterinary instruments and rectal palpation was also suggested as a possible role for biting insects.[3] In reality, the large amount of virus continually shed by PI cattle is considered the most important source of BVDV.[3,93,95]

The BVDV may also be transmitted via infected semen or embryo transfer.[3] An important reason for monitoring the safety and purity of MLV bovine vaccines is that NCP strains, accidentally introduced into vaccines, have induced BVDV disease in susceptible vaccinates. The movement of BVDV in viremic heifers/cows to the developing fetus/oocyte or to the uterus can result in disruption of the fertilized oocyte's implantations in addition to causing developmental defects or PI individuals.

Clinical Manifestations

BVDV infections occur in cattle in various forms. Creating unique disease categories is therefore nearly impossible. The BVDV can cause single organ infections, involve several systems, and/or work in concert with numerous other infectious agents to cause disease. The BVDV has tropism for many organs including respiratory, digestive, lymphoid, reproductive tract, and fetus. Therefore it is overly simplistic to classify infections into specific diseases for BVDV, except for mucosal disease (MD) and PI calves resulting from fetal infections.

The role of BVDV in either synergistic or mixed infections with other agents is most likely due to its well-known immunosuppression. In the review by Potgeiter,[3] there are numerous references to the effects on the lymphoid organs and reduction in B cells, T cells, and neutrophils. Likewise, there may be a decline in T-helper and T-cytotoxic lymphocytes. In addition to immunosuppression in the bovine acquired immune system (humoral and cell mediated), the innate immune system of the bovine respiratory tract can be impaired by BVDV.[96]

Acute Transient Infections

As with many viruses, BVDV infections in the postnatal susceptible calf are most often inapparent. Numerous serologic surveys indicate the presence of antibody-positive cattle in the unvaccinated population. The young calf is usually the target of the BVDV after the loss of maternal antibodies. The mean half-life of passively acquired antibodies for BVDV is approximately 23 days.[16] The actual

age the calf becomes susceptible to infection is thus dependent on the amount of BVDV antibodies absorbed from the colostrum and dose of virus.

Respiratory Disease

BVDV may cause primary respiratory infection.[97-99] Bovine viral diarrhea viruses may also occur in conjunction with other agents such as BHV-1, PI-3V, BRSV, bovine coronavirus, bovine adenoviruses, *M. P. multocida, H. somni,* and *Mycoplasma* spp.[8,9,11,99-103] BVDV infections can occur as inapparent infections in healthy cattle, as well as cause disease during episodes of respiratory tract disease.[9,12,104] The role of PI calves in respiratory disease, particularly in feedlots, is illustrated by the study with PI calves and their effect on calves in adjacent pens. The risk of treatment was placed at 43% due to exposure to a PI calf, and 15.9% of initial cases of respiratory disease were attributed to PI calves.[92] BVDV can be transmitted quite easily following exposure to a PI calf with 70% to 100% of susceptible, nonvaccinated cattle becoming infected with BVDV under feedlot conditions.[12,104] Also, the beneficial effects of BVDV immune cattle were shown where cattle with higher BVDV circulating antibody levels had lower respiratory disease morbidity rates, lower treatment costs, and lower treatment rates.[105]

Recently attention has been focused on the interaction of *M. bovis* and BVDV in Canadian feedlots including animals with joint disease.[102,103] Samples of lung and joint tissues from feedlot animals that failed to respond to antibiotic therapy were tested by IHC for several antigens including BVDV, *M. bovis, H. somni,* and *M. haemolytica*. *M. bovis* was found in 80% of the cases including 45% of the joints and 71% of the lungs. Infection with BVDV was found in more than 40% of the cases. *M. bovis* and BVDV were the most common pathogens persisting in the tissues of animals failing to respond to therapy.

Digestive Tract Infections

The digestive tract disease associated with BVDV may occur in almost any age group from neonate to adult. As described earlier, the postnatal calf after decay of colostral immunity appears most susceptible. The acute form is manifested by fever, anorexia, and depression along with ulcers/erosions in oral mucosa and tongue and, possibly, diarrhea.[3,70] Mixed infections with other enteric agents such as *E. coli, Salmonella* spp., Johne's disease, rotavirus, coronavirus, and cryptosporidia may exist. Lesions may be noted throughout the digestive tract but are not disseminated in all regions of the digestive tract in every case. Ulcers and erosions in the digestive tract occur frequently with the acute forms. Because of the affinity of BVDV for lymphoid tissues, Peyer's patches in the intestine are often involved.

The acute digestive tract diseases are most likely caused by the NCP strains of BVDV with exposure to the virus occurring in the postnatal animal. This is in contrast with MD discussed later.

Thrombocytopenia/Hemorrhagic Form

Another acute form of BVDV is the hemorrhagic syndrome.[106] This form is characterized by thrombocytopenia, bloody diarrhea, hemorrhages on visible mucosa surfaces, bleeding from injection sites, and death.[3,106,107] The NCP strains are the biotype involved. The mechanism of thrombocytopenia and bleeding is not clear. The hemorrhagic form is usually fatal and occurs in both calves and adults. Hemorrhagic syndrome is not believed to be a manifestation of persistent infection with BVDV.

Mucosal Disease

MD was thought by several investigators to be the "classical" form of BVDV with low morbidity and high mortality. MD is the result of a PI calf (by definition infected with NCP strain) developing disease (MD) after infection with a CP strain (closely related to the NCP strain). Infection with a CP strain may occur via exposure to CP strains circulating in cattle, but it most likely occurs when the NCP PI strain mutates to form the related CP strain.[70] The disease is characterized by severe digestive tract disease with ulcers and erosions throughout the tract, skin lesions, and hoof lesions (interdigital). The disease is uniformly fatal. Concern was that MLV BVDV vaccination may have contributed to MD. However, with such a low PI rate (<1% of cattle entering the feedlot) and almost all cattle at the feedlot receiving the MLV BVDV vaccines, there is an extremely low incidence of MD to support that connection to BVDV vaccine. Also, one study demonstrated that vaccination of PI calves with different MLV vaccines did not induce MD.[108]

Reproductive Tract Infections/Fetal Infections

The outcome of infection in the susceptible heifer/cow depends on the stage of the pregnancy. An excellent review of BVDV reproductive consequences gives numerous references to field studies, diagnostic laboratory reports, and experimental studies.[72,109]

Exposure to BVDV in the susceptible female shortly before breeding or early in gestation does have negative effects on conception and/or implantation of the fertilized ovum.[109] The mechanisms for the decreased conception rates are not clearly understood, but they may depend on the time of infection with respect to reproductive stage.[109] The ovary may become infected with BVDV, as has been noted in heifers receiving CP MLV vaccine, acutely infected cattle, or PI cattle.[110-112]

BVDV abortions may also result after infection during the embryonic stage of 45 to 175 days.[109] Other outcomes of fetal infections may occur during this interval, and abortions can occur after this as well. Both NCP and CP strains have been isolated from aborted fetuses.[109] Label indications for several current NCP and CP MLV BVDV vaccines in the United States indicate these products may not be safe in pregnant cows/heifers.[27] With a limited number of exceptions such as females being vaccinated before breeding and/or within the past 12 months, there are a few MLV vaccines approved for use in pregnant cattle.[27] Clinicians and cattle producers should follow the label indications explicitly for each MLV vaccine.

Fetal infections—persistently infected calves. Fetuses infected by an NCP BVDV between days 42 and 125 may survive and be carried to term, be born alive, and survive as a lifetime shedder of virus.[71,87] Not all fetuses exposed in this time frame will result in persistent infection; some may be aborted or develop congenital defect(s).[109] The CP

strains cannot cause PI infections. The PI calf is the most important cattle reservoir of virus, shedding virus in all secretions/excretions. PI calves are immunotolerant to the infecting NCP strain and may respond by developing antibodies to heterologous BVDV strains including MLV vaccine strains.[108] However, infection of a PI calf with a closely related CP strain of virus could lead to MD and the eventual death of the calf.

Congenital effects. The fetus exposed between days 100 and 150 of gestation may develop congenital defects.[109] The defect depends on organ development when infected. A wide variety of defects may occur, with cerebellar hypoplasia in newborn calves the most documented or observed.[109] The affected calves usually die soon after birth or are euthanized. Diagnosis requires examination for gross and microscopic lesions. Other defects may include microencephaly, hydrocephalus, hydranencephaly, porencephaly, hypomyelination, cataracts, microphthalmia, retinal degeneration, optic neuritis, thymic hypoplasia, hypotrichosis, deranged osteogenesis, brachygnathism, and growth retardations.[109] Cerebellar hypoplasia calves have difficulty becoming ambulatory and may be ataxic with other neurologic signs, often resulting in death or euthanasia.[109] Clinicians should be aware of potential congenital defects because a variety of sequelae may occur if BVDV circulates among pregnant females. Not all pregnant females will be in the same gestational stage and, therefore, may not result in a specific outcome.

Late gestation infections. Fetal infections in late gestation may occur after organogenesis is complete and after development of the immune system (last trimester). These infections in the last trimester result in calves born with BVDV antibodies in the precolostral serum and without detectable virus as the fetal immune system clears the virus.[109] The term "congenitally infected" (CI) has been used for these virus-negative, BVDV-seropositive calves (at birth), and they appear to be at greater risk for severe illness than calves born antibody negative.[113] The extent/prevalence of these CI calves remains to be determined in cattle operations. Some years ago, the use of MLV BVDV vaccines in the last trimester (off label) was advocated by some to boost colostral antibodies in the dam. However, reports indicate negative effects of vaccine virus infecting the fetus when the dam was vaccinated with MLV BVDV in the last trimester.

Infections of the Bull

Semen of bulls, either from acutely or PI infected, may contain BVDV.[114-120] Bulls with BVDV in semen may sire calves, but their breeding efficiency may be reduced.[114,118] These bulls and their semen could infect susceptible females.[118,120]

Recently in the United States, BVDV was detected in testicular tissues by PCR up to 7 months after the seronegative bulls were infected with NCP BVDV.[121] The bulls recovered from the acute infection and subsequently became seropositive. Persistent testicular infection will have to be studied further to determine its role in transmitting BVDV to susceptible females. More recently, susceptible postpubertal bulls given an NCP MLV vaccine had prolonged testicular infection after recovery from the acute vaccinal infection.[122] The clinical implication of persisting vaccine virus (NCP) in the testes and its role in fertility remains to be determined. A commercial CP MLV BVDV vaccine protected bulls against the persistent testicular infection when exposed to an NCP BVDV strain.[123]

Diagnosis of BVDV Infection

The definitive diagnosis of BVDV in disease requires extensive use of specific laboratory tests. Although necropsy and histopathology may give strong indications of BVDV, other agents (predominantly viruses) such as foot-and-mouth disease virus, Rinderpest, and MCF may have features similar to BVDV. With keen awareness of exotic diseases, practitioners must consider these diseases along with BVDV as part of foreign animal disease surveillance in the United States. Bovine viral diarrhea virus infections may occur together with other bacterial agents with the pathologic changes often more representative of those other agents.

Lesions caused by BVDV may be present in some, but not necessarily all, affected organ systems.[3,70-73] Animals with digestive tract disease may have ulcers and erosions involving the oral mucosa, tongue, esophagus, rumen, omasum, abomasum, and the small and large intestines. Peyer's patches may be necrotic and hemorrhagic. Skin lesions may be seen in some MD cases with patchy hyperkeratosis around the neck, shoulder, and perineal regions.[70] Erosive lesions in the perineal area, prepuce, interdigital space, and coronary band may be evident in MD cases. Fetal lesions in abortions are difficult to detect because the fetus is often autolytic. Congenital defects are detected by characteristic gross and microscopic lesions.

Virus Isolation

Even with many available molecular diagnostic tests, virus isolation remains a standard laboratory technique. The challenge for viral isolation is to provide definitive answers in a timely manner. Often multiple passages in cell cultures are required. Thus an interval of 2 to 3 weeks may pass before the results are known from the viral isolation attempts. Acutely infected animals are best detected when peripheral leukocytes are collected in blood tubes with anticoagulant. The serums from acutely infected animals had virus in only 38.1% of cattle with virus in the blood leukocytes.[12] Thus the PBL/buffy coat is the preferred blood sample for diagnosis of acute infections using viral isolation. Nasal swabs from cattle with BVDV respiratory disease are often used for submission to the diagnostic laboratory to identify BVDV as an etiologic agent in BRD. In addition to BVDV, other viral agents could be isolated as well from the nasal swabs. Selected organ tissues collected at necropsy can be used as inoculum for viral isolation. Most BVDV are NCP: thus agent identification in positive cell culture cases is confirmed for BVDV antigen by fluorescent antibody tests, ELISA, or immunoperoxidase staining. Neutralization tests with monospecific BVDV antisera or monoclonal antibody are sometimes used. Likewise, CP agents are confirmed by these same tests.

Antigen Detection

Until recently immunofluorescent antibody was used to detect BVDV antigen, especially in necropsy tissues and infected cell cultures. The use of immunofluorescence and other antigen detection systems have been greatly enhanced by monoclonal antibodies. This holds true for various ELISA tests and IHC. Modifications of ELISA tests can confirm BVDV in cell cultures.

An antigen capture ELISA (ACE) test for BVDV has recently been developed and is being used worldwide. Originally designed to detect BVDV antigen in serum from PI calves, it is now used to detect BVDV antigen in the fluid from fresh ear notch samples collected in PBS.

The IHC test is also used for PI diagnosis but additionally can be used to detect BVDV antigen in formalin fixed tissues. The IHC test is widely used to diagnose acute BVD in postnatal cattle and fetal infections in addition to PI animal identification.

Polymerase Chain Reaction

Both reverse transcriptase polymerase chain reaction (RT-PCR) and real-time PCR are available in most diagnostic laboratories for detecting BVDV genomic material.[72] These tests are both sensitive and specific for BVDV. By definition, these PCR tests detect by amplification specific regions of the viral genome of BVDV. The PCR tests are completed in hours compared with several days for viral isolation. The PCR tests are used for bulk milk testing; nasal swab tests; and, most recently, for detecting BVDV in fluid from ear notches of PI calves collected in PBS.

Serology-Antibody Tests

The use of serology for BVDV antibodies is usually performed as a virus neutralization test (VNT) in cell culture. An active immune response to a virus, either as exposure to the agent or by vaccination, is quantitated by a rise in antibody levels. In the VNT a rise of fourfold or greater is an indication of infection. This measurement of the active infection requires an acute and convalescent set of samples 3 to 4 weeks apart. The acute sample should be collected as soon as possible in the disease. Most U.S. diagnostic laboratories use both BVDV1a and BVDV2a viral strains in their VNT. A growing number of laboratories include VNT testing for BVDV1b antibodies because it is the predominant BVDV subtype in several regions.

The use of serology is not helpful when only one sample is available in the postnatal animal. Likewise, paired sera from aborting cows are not rewarding because the infection may have occurred several weeks or months prior. Thus the so-called acute sample in abortion cases would likely already contain high antibody levels, which would not increase in the convalescent sample. When selecting animals to test in a disease outbreak, it is suggested that multiple animals be tested including both healthy and diseased animals.

In fetal infections the collection of a single serum sample may have diagnostic value. If a calf's precolostral serum is positive for antibodies to an infectious agent, it is presumed those antibodies were the result of an active infection by the fetus. Likewise, if fetal fluids are collected from an aborted bovine fetus and are positive for antibodies for the agent, it is considered that the fetus was infected with that agent. These assumptions are based on the understanding that the transfer of maternal antibodies in cattle is via colostrum and not transplacental during pregnancy.

The PI calf will be seronegative to the infecting BVD virus during its gestational life, but it could respond with an active immune response to a heterologous BVDV such as a natural field strain or vaccine.[108] Use of serology cannot be used as the sole criteria for PI status.

Attempts have been made to equate high antibody levels with natural infections as compared with antibody levels induced by vaccination. Experiences of our laboratory have indicated that the two types of exposure (vaccination versus natural infection) cannot be differentiated by antibody levels.[12,104]

Diagnosis of the PI Animal

Control programs for BVDV center on the identification and removal of the major source/reservoir of the virus, the PI animal. Numerous tests for BVD infectious virus and antigen are available. The applications for BVDV testing were summarized in a recent review article.[124] When attempts were made to diagnose PI calves several years ago, the virus isolation test was used with two samples collected 4 weeks apart. The criteria for PI status were that both samples had to be positive for infectious virus. Calves that were only virus positive in the first sample were considered acutely infected.

The presence of BVDV antigen detected by IHC in the epidermis of skin samples such as ear notches has been used as criteria for PI status.[125] These samples were from those submitted for IHC in formalin fixed tissues. Selected diagnostic laboratories may request a second sample for IHC after the first positive IHC test. However, in at least three studies one positive IHC from fixed notches was suitable criteria for PI status.[92,93,126] This approach was confirmed when acutely infected animals were tested by the IHC and found to be IHC negative.[104]

The use of the ACE test detecting BVDV antigen in the fluids of the fresh notches is available now in state diagnostic and commercial laboratories. Recently, a variety of tests were evaluated in PI cattle entering a feedlot.[93] Positive ACE samples were detected in PI calves when compared with concurrent samples for IHC.[93] A limited number of low ACE positives (<5%) were IHC negative. Such low ACE positives should be retested. The ACE test for BVDV was negative in fresh notches in all acutely infected animals.[104] Thus a negative ACE test is expected for acutely infected cattle. In another study of five diagnostic methods for BVDV, only PI calves were positive in skin samples by IHC and ACE test.[127]

In some studies pooling of samples and screening either by ACE in fresh notches or RT-PCR will detect a positive in a pool of several samples.[128-130] The positive pool members were then tested individually by the ACE test to identify the individual positive animals.[129,130] The number of individual samples present in the pooled samples with positive PCR assay results ranged from 50 to 100.[130] Screening cattle using pooled samples is an attempt to reduce the cost of testing. The sensitivity of the ACE test using pooled saline from two notches was less than 100%.[128] Increasing the pool size for the ACE

test will decrease the sensitivity.[128] Likewise, another study reported that pooling with 10 samples, 1 positive and 9 negative (1:10), could result in failure to detect 10% of the PI samples and pooling of 100 samples could result in 50% failure to detect a positive.[131]

Veterinarians electing to test for PI cattle should consult their diagnostic laboratory for the tests being offered. Most state diagnostic laboratories offer both the IHC and ACE on notches. Most laboratories adhere strictly to the U.S. Department of Agriculture (USDA)–approved protocol of one animal, one test for the ACE test. A limited number of state diagnostic laboratories and commercial laboratories offer pooled ear notch testing using PCR.

Prevention and Control

The principles for controlling BVDV are similar to those for other diseases such as BHV-1 and Johne's disease. No antiviral treatments are used for BVDV for PI or acutely infected cattle. This section centers on biosecurity and appropriate use of vaccines.

Biosecurity

The introduction of BVDV into a susceptible herd is often by addition of infected cattle. The principal source of virus is a PI animal. Thus a PI animal such as a bull, cow/heifer, or stocker animal could be the source of infection entering a susceptible operation. Direct fence line contact with PI animals has been reported. PI cattle in a feedlot can affect cattle in adjacent pens.[92] In addition to an untested PI animal entering the herd, a heifer/cow could be BVDV test negative yet acutely infected, resulting in a PI calf. Importantly, all new cattle entering the herd must be tested negative (by IHC or ACE) for BVDV and, if female, remain isolated until they calve, with the calves then tested for BVDV.

If a PI animal is found, it should be isolated from other animals, particularly on breeding farms. The disposition of a PI animal includes euthanasia or feeding until market weight for processing. PI cattle cannot enter the marketing system as unidentified. In selected operations, PI cattle could be fed to market weight. However, although some reach adult age, most die of natural disease by 6 months to a year.

More than 140 vaccines against BVDV are available in the United States for use in cattle.[27] These vaccines are MLV and killed, often in combination with BHV-1, PI-3V, BRSV, *M. haemolytica, P. multocida, H. somni, Leptospira serovars,* and *Campylobacter* spp. Vaccine programs for beef and dairy cattle in the United States routinely incorporate use of one or more doses against BVDV. These licensed vaccines are classified as modified live virus (MLV) for parenteral administration (intramuscular and/or subcutaneous [IM or SC] and inactivated (killed [KV] for parenteral use. The parenteral routes include both IM and SQ; however, several vaccines are approved/recommended for SQ to reduce tissue damage, as stressed in beef quality assurance programs. No USDA-licensed intranasal BVD vaccines are available in the United States. Worldwide there have been attempts to use other technologies for BVD vaccine including viral subunit, DNA vaccines, and, more recently, an equine herpesvirus vector expressing BVDV structural proteins.[132,133]

Cattle respond to the MLV and KV by development of a humoral (B-cell) response with neutralizing antibodies.[12,80,104,134] Passively acquired antibodies from BVDV antibody-positive dams have been shown to provide protection.[135,136] Also, calves fed colostrums from vaccinated dams were protected against BVDV disease.[137] In addition, high maternal antibody concentrations blocked a protective response to MLV BVDV1a vaccine.[138] Vaccination with MLV BVDV vaccine will also induce activated T-cell subsets after vaccination.[139] Cattle can also have an activated T-cell system in situations where the calves have maternal antibodies to BVDV.[140] The total protection against BVDV is not likely limited to either T-cell or B-cell function. One must consider, however, the potential for maternal antibodies to inhibit the active immune response following vaccination. With the half-life of approximately 23 days, the date the calf responds to vaccination will depend on the quality and quantity of antibodies absorbed. One study indicated some calves could retain colostral antibodies up to 299 days based on half-life calculations,[16] whereas other calves maintained antibody levels for a much shorter time (mean of 192 days).

The requirements for U.S. vaccines are set forth in the Code of Federal Regulations (CFR) 113.311 "Live virus vaccines" and 113.215 "Killed virus vaccines." Initially, viral vaccines were evaluated by acute challenge with virulent virus 2 to 4 weeks after vaccination. In recent years there have been additions to the regulations relating to efficacy studies and label claims concerning BVDV vaccines for protecting the fetus. The current USDA APHIS CVB regulations for the BVDV reproductive efficacy and label claims are noted at that website.

The BVDV strains in the MLV and KV are predominantly the CP BVDV1a and BVDV2a strains (Tables 42-1 and 42-2). A limited number of vaccines have NCP strains. Both MLV and KV vaccines induce antibodies to a wide range of BVDV strains including the three predominant subtypes in the United States: BVDV1a, 1b, and 2a.[80,141]

For many years the BVDV vaccines contained only BVDV1a strains. A severe acute disease outbreak of BVDV in North America caused by BVDV2a focused attention on another BVDV subtype.[142] The outbreaks occurred in herds with partial or incomplete BVDV vaccinations. BVDV type 2 disease was identified in beef herds receiving BVDV1 vaccines.[143] Attention was given to whether the BVDV1a vaccines induced protection against BVDV2a. Studies showed protection in cattle receiving BVDV1 vaccine and challenged with BVDV.[137,144,145] Yet there was a drive by some companies to add the BVDV2 strains to their vaccines and receive USDA approval. Today, almost all marketed MLV and KV vaccines in the United States have BVDV1a and BVDV2a strains (see Tables 42-1 and 42-2).

Considerable emphasis has been placed on preventing fetal BVD infections, especially PI calves. Vaccination with the BVDV1a MLV vaccine induced protection, preventing calves from becoming PI when an NCP BVDV1a strain was given to the pregnant vaccinates and nonvaccinates.[146] Eighty-three percent (83.3%) of the calves carried to term were protected from challenge with BVDV1a.

Table **42-1**

MLV Vaccines with BVDV Strain and Genotype/Biotype

MLV	Company	Strain	Genotype/Biotype
Express 5	Boehringer Ingleheim Vetmedica	Singer	1a CP
		296	2a CP
Pyramid 5	Fort Dodge	Singer	1a CP
Pyramid 10		5912	2a CP
Pyramid 4	Fort Dodge	Singer	1a CP
BoviShield 4	Pfizer	NADL	1a CP
BoviShield Gold 5	Pfizer	NADL	1a CP
BoviShield Gold FP5		53637	2a CP
Titanium 5	AgriLabs	C24V	1a CP
		296	2a CP
Vista 5	Intervet	Singer	1a CP
		125A	2a CP
Reliant Plus	Merial	NADL	1a CP
Arsenal 4.1	Novartis	GL760	1a NCP
Jencine 4	Schering Plough	WRL	1 NCP

BVDV, Bovine viral diarrhea virus; *MLV,* modified live virus.

Table **42-2**

Killed Vaccines with BVDV Strain and Genotype/Biotype

Killed	Company	Strain	Genotype/Biotype
Elite 4	Boehringer Ingleheim Vetmedica	Singer	1aCP
MasterGuard 5	AgriLabs	C24V	1aCP
		125C	2aCP
Triangle 4+II	Fort Dodge	Singer	1aCP
BVD		5912	2aCP
CattleMaster	Pfizer	5960	1aCP
Gold		53637	2aCP
CattleMaster	Pfizer	5960	1aCP
4		6309	1 NCP
ViraShield 6	Novartis	K22	1aCP
		GL 760	1a NCP
		TN 131	2 NCP
Respishield	Merial	Singer	1aCP

BVDV, Bovine viral diarrhea virus.

When that protocol was used with the same BVDV1a MLV vaccine and vaccinates/nonvaccinates challenged with BVDV2, there was 57.9% protection against BVDV2 causing PI fetuses.[147] The addition of an MLV BVDV2a component to the BVDV1a MLV vaccine in the previously mentioned studies protected all vaccinated heifers' (challenged with BVDV type 2a during pregnancy) calves against PI, while the BVDV1 vaccinates' calves became PI in 6/18 and 7/19 from heifers receiving one or two doses of the vaccine.[148] Another MLV BVDV vaccine containing types 1 and 2 protected gestating fetuses against both type 1 infection (100%) and type 2 (95%).[149] An NCP BVDV MLV type 1 MLV vaccine protected heifers and their fetuses against both BVDV1 and BVDV2 challenge.[150] A duration of immunity study using MLV vaccine in heifers demonstrated protection 370 days after vaccination, and all fetuses/calves were negative for BVDV.[151]

Most susceptible heifers/cows are exposed to BVDV via direct or close contact with PI cattle. In three studies, one in the United Kingdom[152] and two in the United States, PI calves were used to challenge vaccinates and controls with fetal protection as the goal.[153,154] In the United Kingdom, PI cattle with BVDV1a were used to measure the efficacy of KV vaccine.[152] In that study vaccinated and control heifers were exposed to PI heifers with BVDV1a.[152] Vaccination with the KV BVDV vaccine containing BVDV1a provided protection against the PI heifer challenge.[152] In another study in the United States, MLV vaccine with BVDV types 1 and 2 protected pregnant heifers and their fetuses at 149 to 217 days' gestation against exposure to calves PI with a BVDV 2a virus.[153] A KV BVDV vaccine containing BVDV1a and BVDV2a protected fetuses (but not 100% of fetuses) against infection after exposure to PI calves.[154]

The risk of cattle shedding MLV BVDV vaccine virus and infecting contacts appears minimal. A transient viremia may occur in susceptible cattle for a few days after vaccination with MLV BVDV vaccines.[48,155] No shedding in the nasal secretions of the vaccinates has been noted,[48] nor was there infection of susceptible calves housed with vaccinates.[155]

Vaccination programs for cattle depend on the livestock management systems for the beef and dairy operations. One set regimen is unlikely because management will vary.

Breeding Herds for Beef and Dairy

Annual vaccination of the adult breeding herds for BVDV is recommended as part of a herd health vaccine program. BHV-1 and BVDV vaccines are often given to adult breeding beef herds at pregnancy check. This is a management decision, not related to the pathogenesis of BVDV or BHV-1. Vaccinations at pregnancy check for BHV-1 and BVDV have little or no benefit for the fetus, and in some cases, if the dam is susceptible, the fetus may become infected. Ideally vaccination should occur before breeding to maximize protection against fetal infection. USDA-licensed vaccines, both MLV and killed, are used before breeding.[27] For the MLV, it is generally recommended that the vaccines be given 30 to 60 days before breeding or exposure to bulls in natural breeding operations. Certain MLV virus vaccines can be used safely in pregnant cows and are labeled as such.[27] Generally, these vaccines require vaccination prebreeding and/or within a time frame such as the preceding 12 months. Caution must be given to each vaccine label's inserts for those requirements. The same requirements apply to the vaccination of nursing calves. Some MLV vaccines are approved for nursing calves, but label restrictions should be followed, especially as they relate to the vaccination status of the dam.

Calf Vaccinations in Beef Breeding Operations

Generally, it is recommended that calves receive two doses of BVDV vaccines. These are usually done as part of a preconditioning program for beef calves entering stocker and/or feedlot operations. Two doses are given in that maternal antibodies may persist blocking the first dose[16] or, in some cases, a seronegative calf will not respond to vaccination and does respond after a second dose.[155] The initial dose could be given before weaning such as at branding. Other cattle operations may wait until weaning for the first dose and give the second dose 3 to 4 weeks later. Industry practices in beef are moving toward MLV vaccines, although some veterinarians and producers elect to use KV vaccines. One approach is to use KV for the first dose followed by MLV.[156]

Stocker Operations and Feedlots

Cattle for stocker operations are traditionally vaccinated with MLV vaccines containing BVDV, BHV-1, PI-3V, BRSV, and bacterial immunogens. Some cattle operations and veterinarians will elect-to use KV vaccine in selected highly stressed calves. Likewise, at commercial feedlots, the MLV vaccines are standard vaccination protocol. Most feedlots may use only one dose of the MLV BVDV and other viral components, given at entry, but many will give an MLV BHV-1 at reimplanting.

Calf Raising Units

Heifer raising units and veal operations use both modified live and killed vaccines.

Summary

Bovine virus diarrhea viruses (BVDV) are a group of viruses causing both primary disease and/or acting in concert with numerous other agents to produce disease. The PI animal is the central figure as the principal reservoir exposing susceptible cattle. BVDV disease and its impact on cattle are evident in many management situations, from breeding herd to the stocker and feedlot operations. The emphasis should be to recognize that the BVDV PI begins with infection in the breeding herd. Controlling BVDV fetal infections will reduce and, ideally, eliminate the PI animal. The detection and removal of the PI animal is the goal of BVDV control along with biosecurity and effective vaccination. With the diagnostic tests available to detect PI cattle, BVDV impact on cattle production can be greatly diminished with the removal of these individuals.

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