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Viral Diseases of the Fetus

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BOVINE VIRAL DIARRHEA VIRUS INFECTIONS

Bovine viral diarrhea virus (BVDV) is one of the most commonly encountered and economically important pathogens of cattle in North America. Since the mid-20th century, BVDV has been recognized as a significant cause of disease of the gastrointestinal system. The impact of BVDV on reproduction was not perceived for another 30 years, when the occurrence of persistent infection in imunotolerant cattle was described.

BVDV infections may occur in cattle as acute illness- that is, bovine viral diarrhea (BVD)-or as a generally chronic condition-mucosal disease. When susceptible regnant cattle are infected with BVDV, transplacental infections usually occur. Transplacental infections may lead to embryonic or fetal death and abortion, to developmental defects of organs, or to development of immunotolerance and establishment of persistent Infections. Acute BVDV infections contribute, through inmunosuppression, to causing multifactorial diseases, such as diseases of the respiratory and enteric tracts in sussceptible calves.

Clinical Forms of Infection with Bovine Viral Diarrhea Virus

The clinical form of BVDV infection-inapparent or severe BVD, reproductive failure, persistent infection, or mucosal disease-observed within a herd is dependent on interaction of several factors at the time of infection. These determining factors include the biologic properties of the virus, the age and stage of gestation of pregnant cattle, level of immunity of the herd, and the interplay of stressors.[1]

Acute Infections

BVD is an acute postnatal infection in seronegative, immunocompetent cattle. The clinical severity of acute BVDV infections is variable, but a majority of postnatal BVDV infections are inapparent. Milder forms of BVD are characterized by high morbidity, low mortality, a normal host immune response, and minimal mucosal lesions. Usual findings include pyrexia, nasal discharge, and tran- sient leukopenia. Viremia lasts for 3 to 10 days (acute infections with higher virulence isolates may result in viremia of longer duration) and antibody titers rise slowly for 3 months after infection.[2]

Severe acute BVD outbreaks with marked thrombocytopenia, hemorrhages, and high mortality rates have been associated with infection with high-virulence BVDV isolates.

Acute BVDV infections contribute to causing multi- factorial diseases through immunosuppression. Immunosuppression is mediated by suppression of immune functions through the lymphotropism of BVDV. BVDV lymphotropism results in depletion of lymphocytes from lymphoid tissues. Immunosuppression due to BVDV infection enhances the severity of bovine rotaviral enteritis in calves, in addition to directly causing enteritis.[3] BVDV-induced immunosuppression predisposes calves to development of naturally occurring bovine respiratory tract disease (BRD). Indirect effects of BVDV in causing BRD were demonstrated in experimental bovine respira- tory syncytial virus (BRSV) and BVDV co-infections in which more severe respiratory tract and enteric disease occurred than in infections with either virus alone.[4] Sub- populations of lymphocytes were more markedly altered in peripheral blood and lymphoid tissues from co- infected calves than in calves infected with either BRSV or BVDV alone. Co-infected calves had a reduction in the percentage of T lymphocytes (including CD^{8+} lymphocytes and CD^{4+} lymphocytes) in the thymus and Peyer's patches.[5] An additional finding in these calves was more extensive pneumonia, characterized by caudodorsal as well as cranioventral interlobular edema, emphysema, and bronchopneumonia in caudal lung lobes. By contrast, calves infected with BRSV alone had only cranioventral bronchopneumonia.

Transplacental Infection

Transplacental infection is likely to occur in susceptible, pregnant cattle infected with BVDV. The outcome of transplacental infection is dependent on the biologic properties of the infecting virus, especially the biotype of the virus, and the stage of gestation at the time of infection. The potential outcomes of transplacental infections-embryonic or fetal death and abortion, developmental defects of organs, and development of immunotolerance with establishment of persistent infections-are discussed later in the chapter.

Persistent Infection

Fetal infection with noncytopathic BVDV can result in the birth of calves with persistent BVDV infection. The primary means of producing a persistently infected calf is through transplacental infection after a primary acute infection in a pregnant cow, although persistently infected cows (i.e., congenitally infected) also will give birth to persistently infected calves. Persistently infected animals shed large amounts of virus and are therefore carriers and a primary source of exposure for susceptible cattle.[6] In most instances they do not produce detectable antibodies to BVDV, because they are immunotolerant to the virus. Some calves with persistent infection are stunted or weak at birth, have poor growth rates, and die at a young age.[7] Others appear healthy and survive to maturity. The prevalence of cattle with persistent infection is variable; however, on the basis of sampling of randomly selected herds, it has been estimated that 4% of herds in the United States have persistently infected calves.[8] Such animals are at risk of developing mucosal disease.

Mucosal Disease

Mucosal disease, associated with high mortality rates, occurs sporadically (low morbidity) in cattle that usually are between 6 months and 2 years old but may be of any age. Characteristic clinical manifestations include anorexia, pyrexia, diarrhea, loss of condition, and death.[2] Gross pathologic lesions may include erosive or ulcerative lesions on the muzzle and lips, buccal mucosa, and tongue. Commonly, elongated ulcerative lesions occur in the mucosa of the esophagus. Erosions also may be found on the rumen pillars, reticulum, and abomasum. Enteritis may be evident and may vary in presentation from catarrhal to hemorrhagic to erosive/ulcerative. Peyer's patches and lymphoid tissue in the proximal colon may be hemorrhagic.[9] Thymus atrophy and enlarged peripheral lymph nodes are prominent features.

Biologic Properties of Bovine Viral Diarrhea Virus

BVDV is a member of the genus *Pestivirus*, family Flaviviridae,[10] which also includes border disease virus of sheep and hog cholera virus. Pesti viruses are small, enveloped, single-stranded, positive-sense RNA viruses that are antigenically related.

The host range for BVDV comprises domestic or wild ruminants and swine. Pestiviruses are presumed to persist in the environment for no more than two weeks, and are readily inactivated by common disinfectants. Therefore, virus transmission is primarily vertical or by inhalation or ingestion of material contaminated with infected body secretions and excretions (saliva, oculonasal discharge, urine, feces, semen, uterine secretions, placenta, and amniotic fluid) of infected animals.

Isolates of BVDV vary in their relative virulence potentials, which accounts in part for variability in severity of lesions and clinical disease among different cases." BVDV isolates are divided into two biotypes (groups of viruses with the same genetic composition) based on their ability to induce microscopically visible changes (vacuolization and lysis) in host cells in vitro: cytopathic and noncytopathic. BVDV strains are divided into two genetic groups or genotypes-1 and 2-using gene sequencing techniques and cross-neutralization assays. RNA viruses, including BVDV, are prone to mutate; therefore, BVDV has high potential to mutate in response to selective immune pressure. Mutation is the putative strategy used by BVDV to escape the host's immune response and to persist in the cattle population. Antigenic diversity among field isolates has important implications for development of protective immunity.

Biotypes: Noncytopathic and Cytopathic

The two biotypes of BVDV, cytopathic and noncytopathic, have separate biologic roles[2]; biotype differences are important in disease pathogenesis. Both biotypes of BVDV infect cattle and cause disease, but only the noncytopathic isolates cause persistent infections. Isolates that have the ability to

cause microscopically visible changes in host cells (vacuolization and lysis) are assigned to the cytopathic biotype. Isolates lacking this capability are assigned to the noncytopathic biotype. Cells infected with cytopathic BVDV have an 80-kilodalton (kD) polypeptide that is distinguishable electrophoretically from cells infected with noncytopathic viruses, which do not have the polypeptide.[12] This 80-kD nonstructural viral protein apparently plays a crucial role in replication of cytopathic viruses. Diversity in antigenicity among strains is not discretely separable. No link exists between biotype and antigenicity, and strains that are antigenically distinct overlap both biotypes, so protective immunity afforded by a vaccine is not dependent on the biotype of the vaccine virus.

In the laboratory, the presence of noncytopathic BVDV constitutes a significant quality control issue for workers in diagnostic laboratories as well as for manufacturers of vaccines. This is because noncytopathic BVDV isolates commonly occur in commercial fetal calf sera used to supplement cell culture media used in cell cultures to grow viruses. In the diagnostic laboratory, when noncytopathic BVDV occurs undetected as a contaminant of cell culture, accuracy of diagnostic laboratory assays, such as virus isolation tests and serum neutralization tests, is compromised. Noncytopathic BVDV contamination of modified live virus vaccines during the manufacturing process has represented a significant risk factor since these products were introduced.[13] The potential for contamination of cell cultures with noncytopathic BVDV is a continual concern, because between 20% and 50% of commercial fetal bovine serum lots are virus positive[14] for both genotypes.[15] Fetal bovine serum quality assurance procedures applied before use in diagnostic lab- oratory testing or in cell culture production systems to grow vaccine virus include rigorous virus testing followed by the additional precautionary measure of irradiation or chemical treatment.[14]

Genotypes

BVDV strains are divided into two genetic groups or genotypes using gene sequencing techniques and cross- neutralization assays.[16,17] Genotype 1 isolates are primarily classic laboratory reference and vaccine strains. Genotype 2 viruses are found predominantly in fetal bovine serum, persistently infected calves born to dams vaccinated against BVDV, and the more recently described BVDV strains associated with high mortality and acute and peracute infections involving hemorrhage. Biotype, genotype, and antigenic cross-reactivity vary independently,[18] as do biotype, genotype, and pathogenicity.[19] The antigenic differences between genotype 1 and genotype 2 isolates and the clinical importance of genotype 2 BVDV isolates constitute the basis for the recognition that to be effective, vaccines must provide broad cross-protective immunity against both genotype 1 and 2 isolates.

Outcomes of Fetal Infections with Bovine Viral Diarrhea Virus

BVDV enters the susceptible host primarily by the oronasal route and replicates in tonsils, lymphoid tissues, and epithelium of the oropharynx. Phagocytic cells take up BVDV or virus-infected cells, or both, for transport to lymphoid tissues.[20] Viremia is evident 2 to 4 days after exposure. Viremia in a pregnant female is certain to lead to transplacental infection and fetal infection. The outcome of fetal infections with BVDV is determined primarily by the stage of fetal developmental at the time of infection, and by biotype and virulence of the infecting virus.[21] The stage of development of the evolving fetal immune system at the time of infection plays a major role in determining the outcome of infections.[21] Transplacental infections are particularly damaging during the first two trimesters of gestation and may result in persistent infections, fetal death and abortion, or con- genital developmental defects.[22] Persistent infection in calves is the most significant outcome of fetal infection because of the negative effects such infection has on herd production. Persistently infected calves are the most important source of virus to perpetuate disease within and between herds. Moreover, persistently infected calves usually have poor growth rates and die at a young age. Reproductive failure mediated by abortion and birth of calves with congenital abnormalities also are significant outcomes of fetal BVDV infections that adversely affect herd performance.

Persistent Infections

Persistent BVDV infections may be established if infection of the fetus occurs during the third or fourth month of gestation before immunocompetence becomes established.[21,23] Viremia of the pregnant dam, stemming from either a persistent or an acute infection, is the source of the virus that infects the fetus. Before infecting the fetus, BVDV replicates in the placenta. Persistent viremia develops as a result of fetal immunotolerance and failure to develop antibodies against the persisting virus.[6] Persistently infected calves are carriers because they are viremic and shed virus continuously, and they may spread virus within and between herds. The level of viremia may decline with the development of neutralizing antibody and become undetectable as the animal ages[24] as a result of deterioration of highly specific immunotolerance to the persisting virus.[25] Deterioration of immunotolerance, eventuating in an immune response, may result from development of antigenic-variant viruses within the immunotolerant, persistently infected animal.[25] Persistently infected calves frequently are "poor doers," have reduced growth rates, are more susceptible to common calfhood infections of mucosal surfaces including pneumonia and enteritis, and are at risk of developing mucosal disease.[26,27]

Abortion

BVDV infections of fetuses during the first and second trimesters may cause fetal death and abortion. Third-trimester abortions also have been attributed to BVDV infection.[21] Individual isolates probably vary in their ability to cause abortion. The rate of abortion under field conditions is variable, but abortion rates as high as 40% have been reported after experimental infections on day 100 of gestation.[22]

Congenital Defects

Congenital defects may result if infection occurs during midgestation (100-150 days). Congenital defects associated with BVDV infections may involve the nervous system (microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, and hypomyelination), eye (cataracts, retinal degeneration, optic neuritis, and microphthalmia), immune system (thymic aplasia), integumentary system (alopecia and hypotrichosis), musculoskeletal system (brachygnathism, growth retardation, and arthrogryposis), or respiratory system (pulmonary hypoplasia).[28] The pathogenetic mechanisms for development of defects are not known. Because fetal organs and immune system (inflammatory response) are developing during this stage, direct cell damage by viral infection and destruction of virus-infected cells by the evolving immune system are possible mechanisms.[21]

Late-Gestation Transplacental Infections

The outcome of BVDV infections during late gestation (last trimester) is comparable with that with acute post- natal infections of cattle. At this time the fetal immune system has developed to respond efficiently against BVDV infection. Consequently, transplacental infections during late gestation are not associated with a significant level of congenital defects. Third-trimester abortions have been attributed to BVDV infection.[21] The most common outcome of infections during this period is birth of a clinically normal calf with high levels of precolostral antibodies.[21'22]

Diagnosis of Bovine Viral Diarrhea Virus Fetal Infections

Identification of Persistently Infected Cattle

Persistently infected carrier cattle are identified in herds on the basis of tests conducted in a diagnostic laboratory. The tests include (1) the virus isolation (VI) test, (2) the immunohistochemistry (IHC) test, (3) the polymerase chain reaction (PCR) assay, and (4) the enzyme-linked immunosorbent assay (ELISA).

Virus isolation test. The standard VI test format (macrotest), a highly reliable test,[7,29] is not practical for testing a large herd. The standard VI test may be used to test mononuclear cell preparations (buffy coats) from blood samples collected in tubes with anticoagulants. The cells are washed to limit interference from antibodies, which reduce test sensitivity. An adaptation of the standard VI test is the immunoperoxidase microtiter plate VI assay, which is relatively sensitive and specific and is designed to efficiently test large numbers of serum samples, such as in herd testing programs.[30,31] Blood is collected for virus isolation from calves that are 2 months of age or older, when maternal antibody titers have declined, because maternal antibodies reduce the ability to isolate BVDV from the serum of younger persistently infected cattle.[7,29]

Immunohistochemistry test. The IHC test is conducted on skin biopsy specimens (ear notches) collected from animals of any age, fixed in formalin, and submitted to the diagnostic laboratory.[32-34] The fixed skin specimens are sectioned, stained, and examined for the prescence of BVDV antigen. The IHC test, like the VI test, has excellent sensitivity and specificity.[34] Sensitivity of IHC studies is not affected by the presence of maternal antibody, so calves of any age, including newborn calves, may be tested.[34,35]

Polymerase chain reaction assay. The PCR assay may be used to test individual animals (serum, whole blood, or skin samples) or to screen entire herds by testing pooled samples such as bulk tank milk or pooled serum samples for the presence of carrier cattle.[36] The BVDV PCR assay is highly sensitive, but a potential complication with the assay is lack of test specificity, so that false positive results are possible from nonspecific reactions with contaminating viral RNA (unpublished observation). It is therefore advisable to confirm positive BVDV PCR assay results with VI tests.[35]

Enzyme-linked immunosorbent assay. The ELISA may be used to test individual blood samples for the presence of BVDV antigen. The antigen-capture ELISA compares closely with virus isolation techniques for detection of persistently infected cattle using blood samples routinely submitted for BVD diagnosis. The test format is adapted to a microtiter plate assay, which permits efficient testing of large numbers of serum samples.[31,37]

Aborted Fetuses

Diagnosis of BVDV as the cause of abortion is not unequivocal, because fetal infection may not result in abortion. Therefore, the presence of virus, viral antigen, or BVDV antibody in an aborted fetus does not confirm that BVDV was the cause of abortion.[28] The entire fetus should be submitted to a diagnostic laboratory for complete testing because of the complexity of the factors to be considered in conclusively establishing BVDV infection as the cause of abortion or, conversely, in ruling out BVDV infection as the cause of abortion. Diagnosis of BVDV as the cause of abortion is based on evidence of BVDV infection of the fetus (presence of virus, antigen, or RNA in tissues, or antibody in serum or exudates), in conjunction with clinical confirmation of microscopic lesions, most often in fetuses aborted before 4 months of gestation. Microscopic lesions attributable to fetal BVDV infection include a necrotizing inflammatory reaction with mononuclear cell infiltration in several tissues.[38] Other features may include lymphoid depletion of the cortex of the thymus, precocious development of secondary lymphoid tissue, and peribronchiolar lymphonodular hyperplasia. The cerebellum is affected with necrosis and depletion of cells and infiltration of mononuclear cells. Microfocal lesions may be seen in the oral mucosa and in the skin. Skin lesions are characterized by hyperkeratosis and parakeratosis. BVDV antigen may be deposited in lymphoid tissues and in the cerebellum.[9] Demonstration of rising BVDV antibody titers in paired serum samples from dams may be not be possible. This is because antibody titer may have already increased at the time of abortion because of the time lag from infection of the dam to abortion. Identification of BVDV in a fetus in the absence of lesions provides useful information regarding the temporal occurrence of BVDV within the herd.

Congenital Developmental Defects in Term Calves

Diagnosis of BVDV as the cause of congenital developmental defects in term calves is based on evidence of transplacental BVDV infection in combination with presence of characteristic clinical signs and gross or microscopic lesions. Evidence of transplacental BVDV infection is obtained by culture of BVDV or detection of BVDV antigen or RNA. Antibody in serum collected from a calf before it has ingested colostrum also constitutes evidence of transplacental BVDV infection. Calves born with cerebellar hypoplasia have difficulty standing and exhibit a wide-based stance, and are ataxic. Blindness may result from congenital defects of the eye. Ophthalmic examination may be performed to reveal the presence of cataracts. Calves may be born weak and undersized sub- sequent to fetal growth retardation due to BVDV infection.[28] Gross lesions involving the nervous system, eye, immune system, integumentary system, musculoskeletal system, or respiratory system are described in greater detail earlier in the chapter. It is prudent to exercise caution in attributing these lesions to BVDV without demonstrating virus or viral antibody, because other causes for many of these lesions exist. Unfortunately, the virus frequently is cleared by the time calves are born. Pre- suckle serum is useful for diagnostic applications.

Late-Gestation Transplacental Infections

The presence of BVDV antibody titers in serum collected from normal term calves before they have ingested colostrum indicates that infection occurred late in gestation after the fetus developed immunocompetence.

Screening Herds without a History of BVDV Infection

A herd that does not have a history of BVDV infection may be screened to determine if BVDV infection is active in the herd (i.e., determine if animals with either acute or persistent infections are present). One screening approach that limits the expense of testing, as well as labor requirements, is testing for the presence of anti- bodies to BVDV in a representative subset of nonvaccinated, sentinel cattle[39] that are at least 8 months old.[40] The presence of BVDV antibodies in any of these animals indicates that one or more acutely or persistently infected animals are present in the herd.[40,41] The absence of BVDV antibodies indicates that carrier cattle that shed virus, both acutely and persistently infected animals, are not present-the herd is BVDV-free. PCR assays of the somatic cells of bulk-tank milk is another approach that has been used in dairy herds to screen for evidence of carriers with persistent BVDV infection among lactating cows.[36]

Prevention and Control

The goal of a BVDV control program is to prevent fetal infection to eliminate BVDV-associated reproductive losses and the birth of persistently infected calves. Control of BVDV infection is best achieved by avoiding persistently infected carrier cattle and acutely infected cattle, and by maintaining sound immunization practices. Elimination of persistently infected carriers from the herd is accomplished by testing the herd and by closing the herd to incoming animals that are potentially persistently infected carriers or acutely infected, transient virus shedders. Identification and removal of persistently infected cattle require accurate herd-based diagnostic lab- oratory testing.[42]

Detection and Removal of Persistently Infected Carrier Cattle

Removal of persistently infected cattle from a herd and prevention of reintroduction of persistently infected cattle into a herd are essential herd management procedures because persistently infected cattle are the primary source of infection for a herd. Selection of replacement breeding cattle on the basis of performance effectively eliminates some persistently infected cattle from the herd on the basis of poor growth rates. Other persistently infected animals may be eliminated from herds because of the shortened lifespan sometimes associated with persistent infection in cattle. Some persistently infected cattle, however, may have normal growth rates[21,39] and normal lifespans and accordingly be retained in the breeding herd. Consequently, the importance of using laboratory tests to ensure detection and removal of all persistently viremic cattle from a herd is clearly evident. Seed stock producers, especially breeders of purebred cattle, should be strongly encouraged to test their animals and remove persistently infected carrier animals from their herds.[35]

The greatest proportion of persistently infected animals in BVDV-infected herds are calves younger than 6 months of age.[43] Initially, calves are tested, rather than dams, because if calves are tested, information about the persistent infection status of the calves and about that of their dams is obtained simultaneously. This is because persistently infected dams always give birth to persistently infected calves. IHC testing of skin biopsy specimens (ear notches) is recommended for this procedure. Testing and removal of persistently infected calves from the herd must be completed before the breeding season begins, to prevent contacts of persistently infected calves with pregnant cows, so as to prevent transplacental infection, production of persistently infected calves, and perpetuation of infection within the herd. In addition to testing calves, replacement heifers, cows not calving, bulls, and dams of any calves that test positive must be tested.[35]

Biosecurity

Before implementation of an extensive BVDV testing program in a herd, the potential for re-exposure of a BVDV-free herd to BVDV infection after completion of a persistently infected carrier testing and removal program must be considered. Introduction of BVDV infection into a herd may occur by contact with cattle from other herds or with addition of animals to herds. Requirements for a BVDV biosecurity program are that all purchased cattle be tested for persistent infection status or originate from a BVDV-free herd. Purchased replacement animals should be isolated and tested before being added to the herd to avoid introduction of acutely-infected animals. The offspring of

purchased pregnant replacement cattle must also be tested to confirm their BVDV persistent infection-free status before being added to the herd.[40,44] Seed stock producers are obligated to maintain a BVDV-free herd by maintaining strict biosecurity practices, including testing of all animals in their herd to warrant BVDV-free status.[35]

Vaccination and Immunity

The goal of immunization is to prevent infection of target organs such as the fetus. This is accomplished by inducing both the B and T cell arms of the immune system. Free virus is inactivated by the B cell arm of the immune response, by neutralization of BVDV infectivity by immunoglobulin, and secondarily by aggregating virions and enhancing clearance. Infected cells that have potential to release infectious virus are eliminated by T cells.[45]

Optimally, vaccines should provide broad cross-protective immunity that protects the fetus against all field strains of BVDV. Vaccines do provide significant protection against fetal infection, which will limit reproductive disease, including production of persistently infected calves. Vaccines do not provide absolute fetal protection, however. Results of experimental challenge vaccine studies have shown that no BVDV vaccine can induce complete fetal protection, and birth of persistently infected calves in vaccinated cows has been reported in the field. Consequently, vaccination should not be relied on completely to provide protection against fetal infection. Management practices also should be implemented to identify persistently infected carrier cattle and eliminate them from the herd, and to avoid exposure to BVDV infection.[35,46]

Modified live virus vaccines against BVDV activate systemic and local, humoral and cell-mediated immune responses. Immunity induced by modified live virus vaccine generally is more cross-reactive than that induced by inactivated vaccines. Cross-reactivity is important for BVDV immunity because the potential for antigenic variation exists. Other advantages of modified live virus vaccines include longer duration of immunity and a reduced requirement for repeated administration of vaccine. Disadvantages of these vaccines are immunosuppressive properties and the potential to cause severe fetal anomalies and disease attributable to vaccine contamination with adventitious BVDV. Another disadvantage is the potential for restoration of virulence of the virus during infection. Experimental challenge-exposure studies have demonstrated a reasonable degree of protection against fetal infection using a modified live virus vaccine.[47]

Inactivated vaccines are neither immunosuppressive nor fetopathogenic. Inactivated vaccines also offer the advantage of immunization with minimal risk of infection. In general, disadvantages of inactivated BVDV vaccine may be a need for increased frequency of administration due to a weaker neutralizing antibody response and shorter duration of protection.

Timing of vaccination. The best general recommendation for control of BVDV disease includes avoiding addition of replacement animals that are persistently or transiently infected, avoiding purchase of pregnant cattle, removal of carrier cattle from the herd, and adherence to a vaccination schedule based on use of both modified live virus and inactivated BVDV vaccines. It must be recognized that live, replicating vaccines (i.e., modified live virus vaccines) have certain inherent properties (see preceding discussion of benefits of modified live virus vaccines versus inactivated vaccines) that may enable them to induce more complete protection against transplacental infection. Therefore, it may be wise to recommend vaccination of unstressed, healthy heifers, isolated from pregnant cows, with modified live virus vaccine. All replacement heifers should be vaccinated twice with modified live virus before breeding. In non- vaccinated animals, modified live virus vaccines should be administered three estrous cycles (i.e., 2 months) before breeding. Administration of inactivated vaccines to heifers before breeding should be timed so that maximal responses are achieved. Booster vaccinations should be administered in accordance with the vaccine manufacturer's recommendation.

It may be difficult to time vaccination in a dairy herd to avoid use of modified live virus vaccines on premises with pregnant animals, because dairy cows typically are at various stages in their reproductive cycles. Beef calves, weaned at 5 to 7 months of age, typically are seronegative or have low titers of maternal antibody at weaning time.[7] Thus, calves should be immunized before weaning so that they are protected at weaning when they enter concentration points at which a high risk of infection exists.[46]

BOVINE HERPESVIRUS-1 (INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS) INFECTION

Bovine herpesvirus-1 (BHV-1) is well recognized as a pathogen that infects the respiratory and reproductive tracts and also infects the fetus, potentially leading to abortion. BHV-1 infects cells of the upper respiratory tract, causing rhinitis, conjunctivitis, and tracheitis. Respiratory tract infections with BHV-1 also may contribute to establishment of bacterial bronchopneumonia by impairing host defenses, such as by diminishing lung clearance mechanisms and by immunosuppression. If infection occurs in nonimmune pregnant females, systemic infection, fetal infection, and abortion are the likely consequences. Genital infections may result in development of pustular vulvovaginitis in females or balanoposthitis in males. Genital infections, characterized by formation of variable numbers of small nodules, vesicles, focal erosions, or ulcers visible on inflamed mucosal membranes, occur transiently and resolve spontaneously in 1 to 2 weeks.

Important Biologic Properties of the Virus

BHV-1 is a member of the family Herpesviridae, subfamily Alphaherpesvirinae. In addition to causing a range of clinical diseases, it also can establish latent infections localized in trigeminal and sacral ganglia.[48] Latent BHV-1 can become reactivated under certain circumstances such as stress or after corticosteroid treatment.[48] Vaccination with most modified live virus vaccines has the potential to pro-

duce latent infections. Because of viral latency and reactivation, cattle that recover from BHV-1 infections may serve as a source for future infection of unexposed cattle. BHV-1 is fairly resistant to environmental influences and can survive for 5 to 13 days in warmer environments, but because the virion is enveloped, it is susceptible to most disinfectants.[49] Only one serotype of BHV-1 is recognized.

Infection by BHV-1 is transmitted by direct contact with upper respiratory, conjunctival, or genital tract mucous membranes. Infected animals shed virus from respiratory mucous membranes and secretions, or genital mucous membranes and secretions, for 8 to 16 days after exposure.[49] The virus is present in all fetuses aborted as the result of BHV-1 infection, and these fetuses can serve as a source for transmission of disease.[50,51] Venereal trans- mission and the use of contaminated semen or instruments during artificial insemination are the primary means for transmission of these genital infections. BHV-1 can be isolated from the semen of exposed but clinically normal bulls, and this should be considered when exposed bulls are used either for natural breeding of un- exposed cows or as semen donors.[48]

Fetal Infection and Abortions

When BHV-1 respiratory tract infections occur in non- immune pregnant females, the likely outcomes are viremia and subsequent fetal infection and abortion.[50] Exposure of multiple susceptible animals in a herd can result in abortion storms, with as many as 25% to 60% of cows in a herd aborting.[51-53] Sporadic abortions also may be seen, particularly in herds with a previous history of vaccination or exposure. Abortions also can occur when pregnant cattle are vaccinated with conventional modified live virus vaccines.[54]

BHV-1 abortions may occur at any gestational stage, but naturally occurring abortions are most common between 4 and 8 months of gestation.[51-53,55] Aborting cattle may be subclinically infected or exhibit overt clinical disease. When clinical signs are present in aborting cows, they usually manifest as respiratory tract disease or conjunctivitis.[55] Abortions are rarely seen in conjunction with infectious pustular vulvovaginitis.[50] Abortions often do not occur for several weeks after appearance of clinical signs in the dam. The incubation period in one group of pregnant heifers, experimentally infected intravenously, ranged from 17 to 85 days.[56] The mechanism for this latent period between maternal exposure and abortion is unknown, although some evidence suggests that the virus may reside in the placenta for extended periods before infecting the fetus proper, without causing abortion.[51]

Diagnosis of Abortion

BHV-1 infection of the fetus results in rapid fetal death (24-48 hours), but expulsion is delayed for up to 7 days, with consequent autolysis of tissue of variable degree. The placenta often is retained. Gross changes in the aborted fetuses often are obscured by autolysis, but tiny (1- to 3-mm-diameter) white-tan foci may be evident on the surface of liver and lung. Red-tinged serous fluid in body cavities and red color of fetal tissues reflecting the autolysis usually are evident. The placenta may be edematous.[52]

BHV-1 abortions can be confirmed by immunohistochemical tests and microscopic examination of fetal tissues. Microscopically, scattered foci of necrosis in several organs may be present, which can lead to a presumptive diagnosis of BHV-1 abortion. In particular, foci of necrosis may be found in the liver, spleen, adrenal glands, lung, kidneys, and placental cotyledons. Herpesviral intranuclear inclusions may be present in cells adjacent to necrotic foci but usually are masked because of autolysis. Little or no inflammatory cell infiltrate is found in fetal tissues, reflecting the rapidly lethal effects of BHV-1 fetal infection. The diagnosis can be confirmed either by detection of BHV-1 viral antigen or viral nucleic acid or by isolation of the virus from fetal tissues. Viral isolation may be difficult, depending on the degree of fetal autolysis; placental cotyledons are the preferred tissue for virus isolation attempts if autolysis of fetal tissue is extensive.[51]

The BHV-1 antigen can be detected in cryostat tissue section-fluorescent antibody tests (especially kidney and adrenal) and from paraffin-embedded formalin-fixed tissues (especially liver, lung, kidney, adrenal, and placenta) by immunohistochemistry studies.[57] Viral nucleic acid has been detected in aborted fetuses by in situ hybridization[58] and by PCR assay.[59] Determination of BHV-1 antibody titers on paired maternal serum samples is of little help in diagnosing BHV-1 abortions. Most abortions occur several weeks after infection of the dam, so increases in antibody titers will have occurred before abortion. Maternal antibody titers indicate exposure, but without demonstration of rising antibody titers, it is not possible to confirm recent BHV-1 infection.

Prevention and Control

Prevention and control of BHV-1-induced abortion in herds are achieved primarily by implementation of sound biosecurity practices and by vaccination. Biosecurity practices should include control of movement of new stock into a breeding herd to eliminate the possible introduction of BHV-1 into a susceptible herd. Screening of semen used in artificial insemination for BHV-1 contamination and selection of seronegative donor bulls are recommended measures to prevent venereal transmission.[60] Eradication programs have been established in certain European countries, such as Switzerland, Denmark, and the Netherlands.[61]

Vaccination commonly is practiced to prevent and control BHV-1 infection because of the high prevalence of BHV-1.51 Numerous BHV-1 vaccines are commercially available and include both modified live virus and inactivated vaccines. Manufacturers' recommendations should be followed to achieve optimal immune responses and to avoid potential adverse effects associated with their use. Most modified live virus vaccines can cause all of the manifestations of BHV-1 infection in the bovine reproductive tract.[62] Because of the risk of vaccine-induced abortions and exposure of susceptible dams, most modified live virus vaccines are not recommended for use in pregnant cattle or

calves suckling pregnant cows. Inactivated vaccines are safe for use in pregnant cattle. If modified live viruses are routinely employed, the timing of injections can be scheduled to reduce the risk of vaccine virus-induced abortion or transmission of virus to susceptible animals. In beef herds, heifers may be vaccinated before the beginning of breeding season. In dairy cattle, the simplest means of reducing such risk is to vaccinate heifers only before breeding (4-6 months of age, and again at 8-12 months) and to vaccinate postpartum heifers and cows at the time of routine postpartum examinations. These animals are not pregnant and tend to be housed with like animals. Furthermore, routine vaccination should result in development of a "herd immunity," so that likelihood of transmission of vaccine virus to susceptible cattle is reduced.[63]

BLUETONGUE VIRUS INFECTION

Bluetongue virus is an orbivirus that infects cattle and sheep; in North America, it is transmitted by the biting midge, *Culicoides variipennis*.[64] The midge becomes persistently infected with bluetongue virus and may transmit virus for several weeks.[65] Bluetongue virus infection of cattle is common in endemic areas of the world, which correspond to the geographic distribution of the vector. Bluetongue virus is not contagious, and vertical transmission is not important, so perpetuation of the virus in nature is dependent on continuous cycling of virus between the insect vector and susceptible ruminant animals.[66] Cattle are considered to be natural reservoir hosts of bluetongue virus. Although bluetongue virus is common in cattle in endemic areas, bluetongue disease is rare.[67] After onset of infection, cattle may be viremic for up to several weeks, during which time infected animals may act as viral amplifiers and reservoirs for the transmitting *Culicoides* vector.[65] Although viremias may be prolonged, they are not persistent, nor does this agent cause immunotolerance.[67] Prolongation of viremia in cattle, which facilitates infection of the insect vector, probably is the result of the strong association of bluetongue virus with erythrocytes, which may protect virus from elimination by neutralizing antibody.[65]

Fetal Infection and Abortions

Bluetongue virus fetal infection of cattle and sheep can occasionally result in abortion, but teratogenesis is more common.[68] Naturally occurring bluetongue virus fetal infection and abortion have been reported in cattle, but only in countries in which modified live virus vaccines have been used. Vaccine strains of bluetongue virus are the likely cause of naturally occurring fetal infection, because studies in pregnant sheep have shown that bluetongue virus crosses the placenta and produces fetal malformation only after the virus has been altered by adaptation to cell culture.[68]

When rare fetal infections with bluetongue virus do occur, they mimic results from experimental infections. Infection of susceptible heifers or cows during early gestation (during the first 90-100 days of gestation) may result in fetal death (by either resorption or abortion). Fetal infection between 75 and 100 days of gestation results in stillborn fetuses or in the birth of weak calves or calves with cerebral malformations.[69] Central nervous system abnormalities due to bluetongue virus infection in term calves may include hydranencephaly or cerebral cysts.[67-69] Cerebral malformations do not occur in fetuses infected after 150 days of gestation. These late-term fetal infections may have no effect on gestation or may result in premature births. The infected calves may have no visible lesions or a mild nonsuppurative encephalitis.

Diagnostic and Control Considerations

In most instances, infected fetuses surviving beyond midgestation have detectable fetal or precolostral antibodies to the bluetongue virus, but the virus is no longer present.[67-69] The virus is difficult to isolate, and freezing of infected tissue destroys the virus. The virus is rarely if ever isolated from term fetuses if infection occurred before 150 days.[69] Virus may be present in the semen of seropositive bulls, but only when they are viremic. Bluetongue virus in semen is associated with the presence of contaminating erythrocytes or mononuclear cells that carry virus; the virus is not found in spermatozoa.[67] International regulations prohibit movement of livestock and germplasm from countries harboring animals with bluetongue viruses to countries considered virus-free.[67] U.S. livestock industries incur significant losses each year because of these trade restrictions, even though supporting evidence for transmission of bluetongue vims between countries, by either semen or embryos, is lacking.[67]

Vaccines are available, but owing to the rare occurrence of fetal infection or clinical disease in cattle, their use even in endemic regions seems unwarranted. Cows develop immunity to the infecting serotype but remain susceptible to infection by other serotypes.[69] Because bluetongue virus is transmitted by *Culicoides* spp., the modified live virus in the vaccine also may be transmit- ted among animals by the insect vector; thus, if vaccines are used, administration of vaccines should be limited to times of the year when vectors are inactive.[67]

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