Persistent Infections and Immunological Aspects of BVD Virus in Beef Cattle

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Introduction

Bovine viral diarrhea (BVD) was first described in 1946 in New York dairy cattle (1). Bovine viral diarrhea virus (BVDV) is a common infection of cattle with serum neutralizing antibody present in 50 to 90 percent of the world population (2,3).

BVD has manifested itself in a wide variety of clinical entities (4-6). This variability has perplexed producers, veterinary practitioners and researchers for years. Recent findings associated with persistent infections have further demonstrated the complexity of the disease (7-9). An understanding of these findings is important in assessing the effects of clinical BVD. This paper summarizes for the practitioner, the current information about persistent infections and other immunological aspects of BVD.

Etiology

The BVD virus (BVDV) is an RNA virus belonging to the genus Pestivirus, family Togaviridae. These viruses are spread by inhalation or ingestion of material which has been contaminated by infected nasal discharge, ocular discharge, saliva, urine, or feces (3, 10). This virus probably invades the reticuloendothelial system initially with subsequent invasion of mucosal tissues (11, 12).

Viruses isolated from clinical cases of BVD have been differentiated into two biotypes in the laboratory. These are cytopathic (CP) virus which kills infected cells in cell culture, and noncytopathic (NCP) virus which does not (13).

A number of strains of the virus has been identified. These strains are antigenically related and cross-react. The extent of cross-reaction and resulting immunologic cross-protection has not been completely determined (10, 11, 14, 15). Some vaccine trials have indicated that BVDV strains are cross-neutralizing (16-18). Strain differences in pathogenicity have also been reported (7). In another study pneumopathogenicity differed between two strains (19). Different antigenic strains also have diagnostic implications and it has been advised that a broad spectrum of antigenic variants should be included in vaccines due to the antigenic differences in BVDV strains (14). Additional research in this area is warranted and underway (20).

Persistently Infected State and Immunotolerance

A major contribution to understanding BVD has come from the identification of the persistent carrier state which results in immune tolerance of these animals (7-9, 21-24). This information has provided an explanation for the occurrence of many of the clinical complexities associated with BVD.

Pathogenesis

The persistently infected state occurs as a result of placental transmission of NCP virus resulting in infection of the fetus at a particular stage of gestation. Researchers have produced persistently infected calves by inoculation of NCP virus into 58 to 125 day old fetuses in seropositive cows (7). Also, inoculation of seronegative cows 42 to 114 days into gestation produced clinically normal, persistently infected calves (7). In another study, persistently infected calves were produced following the introduction of an apparently persistently infected heifer into a susceptible herd of cattle (25). Only cows that were 81 days or less in gestation at the time of introduction of the heifer produced persistently infected calves. Done, *et al.* produced a variety of congenital defects including persistent infection following inoculation of seronegative pregnant heifers at day 100 of gestation (26). From these and other studies, it appears that the time of greatest fetal risk for persistent infection is approximately 1½ to 4 months of gestation (7,25-28).

A characteristic of the persistently infected animal is its immune tolerance to the strain of virus with which it is infected. These animals are often born and grow normally. In general, fetuses are capable of mounting an immune respone to BVDV at or near 4 months of gestation (8, 29). This time period follows the established time of development of persistent infections.

Incidence

In a study of 3,157 cattle involving 66 beef and dairy herds located in midwestern and western states a frequency of 1.7% persistent infection was reported (30). Some herds were selected because of previous histories of BVDV infection. Six of the 66 herds sampled (9.1%) had persistently infected animals. Percentages of persistent infected animals ranged from 0% to 27% in the herds sampled. Another study involving 3,151 animals in England and Wales reported that 1.8% of animals were viremic, although animals were not proven to be persistently infected (31). Veterinarians should be aware of the potential for large numbers of animals to be persistent carriers of BVDV.

Outcome

The outcome of persistently infected fetuses is variable. Fetuses infected by NCP virus in utero do not necessarily survive to birth or survive the neonatal period. Persistently infected animals have no neutralizing antibody to BVDV at birth and continue to shed the virus following birth (7,8,22,26,32). The neonatal mortality of persistently infected animals is often higher than in normal populations of calves (8,32). Calves may be weak with limited survivability compared to apparently normal healthy calves that survive to maturity (8, 30,32). Apparently healthy, persistently infected animals have been shown to have lesions of glomerulonephritis and encephalitis, indicating that even though perceived as healthy, the NCP virus does have some adverse effects (33,34). Other persistently infected animals had low serum protein levels which gradually increased from birth to 12 months of age (35).

Many of these animals die of muscosal disease caused by superinfection of field strain CP virus (9,21,24,33). In one study, only one of eight animals inoculated survived. Two of the eight animals did seroconvert to BVDV with titers of 1:16 and 1:64 but the others remained seronegative. In another trial, persistently infected cattle were not protected from challenge with CP virus following vaccination with one of two strains of MLV vaccine or a KV-BVDV vaccine (36). Persistently infected steers given MLV had higher antibody responses and longer intervals to development of mucosal disease. Persistently infected steers given KV vaccine developed clinical signs of mucosal disease as quickly as nonvaccinated persistently infected controls.

Fetal infection with CP virfus has been reviewed (4). CP virus has been shown to produce abortion, congenital defects, and stillbirths. However, it has not been shown to produce persistently infected animals in which mucosal disease can be produced.

Forms of Immunity

Active-Normal Animals

Exposure of normal, susceptible, seronegative animals produces an immune animal that does not carry BVDV. However, multiple alterations of immune function following BVDV infection have been reported (2,4,15). Infection with BVDV results in a leukopenia, especially with decreased lymphocyte function. Decreases in number and function of both B and T lymphocytes were seen in cattle inoculated with CP-BVDV (37). Neutrophils and monocyte also are depressed in number and function in BVDV infections (38). Lymphoid depletion is a frequent necropsy finding.

The predisposition of concurrent or subsequent disease following BVDV exposure has been studied (39-45). Reggiardo and Kaeberle concluded that BVDV infections depressed normal defense mechanisms, presumably humoral factors or phagocytic function, resulting in uninhibited systemic circulation of bacteria during infection (39).

Active—Persistently Infected Animals

Persistently infected animals are seronegative at birth, usually remain seronegative, and have altered cellular and humoral immune function (46-49). Cattle persistently infected with NCP virus had altered lymphocyte function and neutrophils had significantly impaired capability to ingest *Staphylococcus aureus* (47). There are indications that the perceived lack of immune response to NCP-BVDV in persistently infected animals is not complete. Renal glomerular lesions in persistently infected animals are believed to have an immunologic origin (34).

NCP-BVDV persists and replicates in blood leukocytes. In mononuclear cells, BVDV has been found in B and T lymphocytes, monocytes, and in null cells (48). Null cells are a group of cells which appear to be immature T-cell lymphocytes (50). Bolin, *et al.* found 4.4% of all mononuclear leukocytes to be infected with NCP virus in persistently infected cattle (48).

Passive Immunity

Levels of passive antibody transferred to the newborn in colostrum are important because maternal antibodies do not cross the placental barrier in the cow. Maternal antibody titers to BVDV tend to decrease at the rate of half their remaining titer approximately every 20-21 days (51,52). Duration of maternal antibodies to BVDV ranged from 95 to 231 days depending on the initial titer (51). In another study involving nine beef calves with an average initial titer of 1:692 at two days of age, maternal antibody declined to 0 by 200 days of age (36). In general, the maternal antibody to BVDV decreases enough during the first 6-8 months of life to allow a calf to become susceptible to infection (51,53,54). With the disappearance of maternal

antibody, BVDV titers of calves increased from natural exposure (53,54).

There are indications, however, that calves are able to develop active immunity to MLV-BVD vaccine virus prior to loss of all passive immunity to BVDV. Calves serologically converted to MLV-BVD vaccine virus when maternal BVD antibody titers were between 1:20 and 1:96 at 84 days of age (51,52).

Interferon Release

A study has evaluated the production of interferon in BVDV infection in both fetuses and adult cattle (55). This study concluded that no differences existed in the ability of the adult or the fetus to produce interferon. Thus, deficient interferon may not be a basis for enhanced susceptibility of the fetal host to viral infection. In dams inoculated at 95 or 149-150 days of gestation, cows produced circulating interferon between days 2 and 9 and fetuses produced circulating interferon from days 13 to 21 post-inoculation.

Diagnostic Application

Correct interpretation of diagnostic laboratory results is extremely important in the diagnosis of BVDV infection. (56). Paired sera are required for reliable serologic diagnoses (57,58). Obtaining valid serum samples is not always possible in cases of abortion due to the length of time between infection and abortion. Antibody titer may have already increased by the time of abortion (6).

Isolation of NCP-BVDV, CP-BVDV, or both by a diagnostic laboratory is the most definitive diagnosis available. Bovine fetuses born normally or aborted have about a 10% infection rate with NCP-BVDV (59). Serologic diagnosis using serum-virus neutralization and tissue examination by immunofluorescence techniques (fluorescent antibody) are also frequently used (6,56). BVDV infections have also been detected successfully, using the direct immunofluorescence technique on nasal epithelial cells collected on cotton swabs (60).

Identification of the persistent carrier state on a herd basis is an important consideration in BVDV diagnosis (6,49). Serological identification of the persistently infected animal is not possible on either an individial animal or herd basis without knowledge of the exposure status of the animal and or herd. Viral isolation is required to confirm exposure. The NCP-BVDV from persistently infected cattle survived equally well in serum, plasma with EDTA, or plasma with heparin for up to five days (61,62). Samples were stored in the dark at room temperature. In another study, NCP-BVDV from persistently infected calves was readily isolated from serum, plasma, or cell lysates after freezing and thawing from persistently infected calves (48). Viral isolation on a herd basis is usually not practical to identify carrier animals but could help eliminate the possibility of family lines becoming infected due to transplacental transmission. (49)

Summary

Understanding persistent infection and other immunological considerations of BVDV infection is important clinically for diagnostic purposes as well as in design of effective control programs.

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