INFECTIOUS BOVINE RHINOTRACHEITIS, PARAINFLUENZA-3, AND RESPIRATORY CORONAVIRUS

Sanjay Kapil, DVM, PhD,
and Randall J. Basaraba, DVM, PhD

Bovine respiratory disease (BRD) complex is caused by a variety of viral, bacterial, and fungal pathogens. Economic losses from BRD in the United States have been estimated to be $640 million annually, and[46] this is a more serious disease in stocker and weaned cattle than in yearling cattle. Viruses commonly involved in BRD are infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and parainfluenza-3 (PI-3) virus. Bovine respiratory coronavirus (BRCV) is an emerging pathogen causing upper and lower respiratory tract disease in feedlot cattle, and clinical disease occurs when the animals are stressed, such as during shipment.[48]

INFECTIOUS BOVINE RHINOTRACHEITIS

Role of IBR in BRD

As a result of IBRV infection, necrosis of the epithelium of the respiratory tract compromises ciliary clearance of bacteria of the upper

From the Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas

respiratory tract. This leads to increased colonization and bacterial growth in the respiratory tract preceding bacterial bronchopneumonia. The virus infection also leads to induction of a variety of cytokines, some of which have antiviral activity, such as interferons. Other cytokines may be beneficial or detrimental to further colonization of the respiratory tract with bacteria. Divalent cations, such as zinc and iron, are increased following respiratory tract infection with viruses, and these changes influence adherence of the bacteria, production of adhesins, and the immune responses, and favor colonization of the lungs by bacteria and subsequent development of pneumonia. Viral influences can alter surfactant and fibronectin production, and thus affect bacterial colonization. Thus, a variety of factors act in concert to increase the colonization of the respiratory mucosa with pathogens.

Alveolar macrophages are the first line of defense against invading microorganisms. Infection with bovine herpes virus (BHV)-1 impairs macrophage, polymorphonuclear neutrophil (PMN), and lymphocyte functions. Thus, impaired macrophage function leads to increased respiratory infections. The PMNs are crucial in the clearance of bacteria from the lower respiratory tract. Any delay in recruitment of PMNs because of stress or viral infection allows the bacteria to overwhelm the local defense mechanisms of the lungs.

Properties of IBRV

The IBRV has 162 capsomeres arranged in an icosahedral symmetry. The nucleocapsid is surrounded by an electron-dense tegument.[52] These structural features and size help to identify the virus on transmission electron microscopy. Isolates of IBR cannot be differentiated serologically; however, they can be differentiated by restriction enzyme analysis of the viral DNAs. Three genotypes of IBRV (1, 2, 3) have been identified. Subtype 1 causes infectious bovine rhinotracheitis, subtype 2 causes infectious pustular vulvovaginitis, and subtype 3 causes neurologic signs. The genome of the IBRV is about 135 to 140 kilobase pairs,[34] and the virus makes 54 transcripts (mRNAs).[55] Definite gene assignments are available only for a few IBR genes. The IBRV has 70 proteins, of which 33 are structural proteins.[38] Ten glycoproteins have been identified in IBRV,[53] and have homology with herpes simplex virus 1 glycoproteins.

Epidemiology

The primary portal of entry for respiratory IBRV infection is the nasal cavity. Aerosols laden with the virus are inhaled, and can initiate respiratory infection. Thus, most infections occur when an infected animal is introduced into a herd or when animals are confined in crowded pens in feedlot situations. The main source of genital disease is venereal transmission, and in bulls the infection can be subclinical.[54] Most bulls

for artificial insemination are required to be seronegative for IBRV. In carrier animals the virus could persist in the trigeminal ganglia in a latent stage. If an animal is sufficiently stressed, as during shipping, the virus may become reactivated, with the host becoming viremic again, and virus may be shed into the environment. Infections by IBR start as foci with the herd of infection and spread within 2 to 4 weeks throughout the herd; the outbreak ends in 4 to 6 weeks when all animals have been exposed to the virus. Most infections occur in animals over 6 months of age, after maternal antibodies have waned. Both humoral and cell-mediated immune responses provide protection against the disease. Wild ruminants, such as deer, have been reported to be reservoirs of the virus. Other ruminants, such as goats, are also susceptible and can transmit the virus to domesticated cattle. Thus, cattle from endemic areas should be considered as carriers and capable of transmitting to previously unexposed animals. The severity of clinical disease is related to the strain of the virus, the immunologic status of the animal at the time of the infection, environmental stressors, and the age of the animal. The presence of even a low level of passive antibody in neonates is sufficient to reduce virus replication and the severity of the IBR infection. Even modified live IBR vaccines are not innocuous, and can cause death and generalized disease and death in young calves.[8] Face flies can retain BHV-1 for a short time, but they are not involved in either mechanical or biological transmission.[20]

Latency

Latent infection in cattle can be produced by IBRV, even by attenuated vaccine virus. The virus may travel from the sites of initial local infections by axonal migration to the sites of latency, such as trigeminal ganglia and sacral ganglia.[1] A variety of factors, such as host immune system, have been implicated in regulating the establishment of IBRV latency. During the latent stage, limited transcription occurs from a small region of the IBRV genome.[42] The latency-associated transcripts have been mapped to 0.740 to 0.748 map units, and about 1.16 kb fragment of genomic DNA has been reported to be involved in latency-associated transcription[27]; however, the precise feedback mechanisms that initiate latency have not been completely determined. Latent infections can be reactivated by stress, transportation, and parturition.

Pathogenesis

Clinical Signs

Respiratory infection with IBRV varies in severity, depending on the host and the strain of the virus involved. The symptoms can range from no signs to mild signs to severe disease, with about 10% mortality

in severe outbreaks in feedlots. The damage is mainly limited to the respiratory tract with lesser damage to the alimentary tract. High fever (105–107°F), inflammation of nostrils, erosion of nasal mucosa, lacrimation, and conjunctivitis are observed commonly. The inflamed nasal mucosa is the source of the common name of "red nose." If exudate blocks the nasal passages, the animal may develop dyspnea and mouth breathing. Other signs, such as bloody diarrhea, abortion, and vulvovaginitis, indicate other forms of IBR infections in cattle.

Immunity

Following BHV-1 infection the antibodies produced have specificity for the three major surface glycoproteins (gI [gB], gIII [gC], and gIV [gD]) of the virus.[3] After secondary infections the responses to minor glycoproteins, such as gE, become detectable. Following passive transfer (colostrum and monoclonal antibodies) the antibodies prevent death but do not provide complete protection.[33] Antibodies against BHV-1 participate in neutralization and antibody-dependent, cell-mediated cytotoxicity, and also block immunosuppressive effects induced by BHV-1 on T cells.[19]

Interactions with cells of monocyte/macrophage lineage occur early in the course of IBRV infection, and play an important role in all aspects of host-virus interactions. Monocytes and macrophages collected from infected animals can produce IFN-α, function as cytotoxic cells, and express increased major histocompatibility complex (MHC) class II molecules, and thus are functional during the infection. Cytotoxic activity, increase in MHC class II expression, and cellular infiltration have been demonstrated in the lung parenchyma and contribute to pathological changes associated with the disease.[7]

Autologous fibroblasts infected by BHV-1 stimulate a strong gIV (gD)-specific T-cell response. In the supernatants of BHV-1–stimulated leukocytes, IL-2 and IFN-γ have been demonstrated. These cytokines could be essential in recovering from BHV-1 infection.[11] IL-2 has been shown to expand CD8+ cytotoxic T lymphocytes (CTLs) and induce lymphokine-activated cells capable of limiting the BHV-1 infection. Cells capable of producing IFN-γ in response to BHV-1 infection have been recovered from peripheral circulation as early as 3 days after infection. IFN-γ induces MHC class II expression and cytotoxicity in the lung parenchyma.[12] During the early stages of BHV-1 infection (1 to 4 days) a marked reduction in lymphocyte activity occurs. Thus, balance between opposing responses may be critical for the outcome of the disease. Natural killer (NK)-like activity mediated by lymphocytes lacking markers for T or B cells or macrophages is antigen dependent. Bovine NK-like cells can lyse gI (gB) and gIV (gD), but not gIII (gC)-transfected cells.[39] Polymorphonuclear neutrophils play a role in the immunity and pathologic changes associated with BHV-1 infection. In vitro, PMNs treated with BHV-1 antigen produce an interferon-like substance called *polyferon*[43]; however, polyferon has not been demonstrated after in vivo infection with BHV-1, and in vitro interactions of PMNs with other

bovine viruses, like PI-3, BRSV, and BVD, fail to induce this protein.[6] PMNs from BHV-1–infected animals, however, have reduced chemotactic and phagocytic activities. Selective depletion of PMNs ameliorates lesions of fibrinous pneumonia with *Pastaurella haemolytica*.

Gross Lesions

Gross lesions of uncomplicated IBR infections are restricted to the muzzle and upper respiratory tract, including nasal cavities, nasopharynx, trachea, and primary bronchi. The lungs are normal, although there may be secondary bronchopneumonia or interstitial emphysema from labored respiration as the result of upper and lower airway obstruction. There is swelling and congestion of respiratory mucosal surfaces with petechiae, serous to catarrhal nasal discharge that becomes mucopurulent following secondary bacterial infections. Severe cases have profuse fibrinopurulent inflammatory exudate in the upper airways that form friable pseudomembranes that overlies foci of necrosis and mucosa that is denuded of epithelium. Fibrinonecrotic exudate becomes encrusted over the muzzle in severe cases. Excessive exudate may block airways, resulting in respiratory distress. Regional cervical lymph nodes become swollen and exude edema fluid when sectioned.

Conjunctivitis can occur concurrently with the respiratory form of IBR, but may also occur as the primary clinical manifestation. Conjunctiva are bilaterally reddened and swollen by edema fluid and inflammation, resulting in excessive lacrimation. Clear ocular discharge progresses to mucopurulent following secondary bacterial colonization.

Histopathology

Microscopic changes of IBR infections reflect the gross lesions. Rhinitis, laryngotracheitis, and bronchitis is characterized by serous to mucopurulent and fibrinous exudate with epithelial necrosis, erosions, or ulceration with hemorrhage. Hyperemia is accompanied by neutrophilic and mixed mononuclear cell infiltrates. Acidophilic intranuclear viral inclusions in respiratory epithelium are present in the febrile phase for 2 to 3 days following infection, and are therefore of little diagnostic value. Viral inclusions are more often seen in experimental infections, and are best demonstrated with acidic fixatives.

Laboratory Diagnosis

Laboratory testing can confirm the clinical diagnosis of IBR based on characteristic signs of disease, gross lesions of the upper respiratory tract, epidemiology of the outbreak, and a clinical history of abortions or other IBR-related clinical signs. Laboratory diagnostic methods include serology, virus isolation, and direct fluorescent antibody test.

Serologic diagnosis of IBR is based on the increase in antibody titer

to IBR virus in serum samples collected during the acute stage of the illness and 2 to 3 weeks postinfection. The presence of preexisting viral titers from immunization, natural exposure, or colostrum precludes interpretation of results from single serum samples. The serum neutralization test is the most common serologic test, and results are available in 3 days.

The virus is easily isolated from clinical specimens, and produces characteristic cytopathic effect in cell cultures. Nasal and conjunctival swabs collected early in the febrile stage of the illness are the specimens of choice. Virus may be isolated from postmortem specimens collected following gross necropsy examination. Those samples can include mucosa from the nasal cavity, larynx, trachea, and lung, including large airways. Viral antigens may also be detected in postmortem specimens by fluorescent antibody techniques. Molecular biology polymerase chain reactions (PCR) may eventually provide additional rapid diagnostic methods for IBR in latently infected animals and in semen samples.

PI-3 VIRUS

Role of PI-3 in Bovine Respiratory Disease

Shipping fever is a major cause of morbidity and mortality in recently shipped cattle. PI-3 is the most consistent viral isolate, but concurrent infections with bovine diarrhea virus, IBRV, adenoviruses, bovine respiratory syncytial virus, *P. hemolytica* or *P. multocida*, and *Mycoplasma* spp are common. Increased susceptibility to bacterial infections in shipping fever is due to virus-induced dysfunction of the host respiratory defense mechanisms.

Parainfluenza virus type 3 (PIV3) is a ubiquitous paramyxovirus with a worldwide distribution. Uncomplicated PIV3 infections is not an important cause of death in calves, but alone causes mild to inapparent clinical signs.[10, 16] The importance of PIV3 is in mixed infections as part of the bovine respiratory disease complex of feedlot cattle. PIV3 is commonly isolated from lungs of cattle in which *P. haemolytica* is a copathogen. The mechanism by which PIV3 predisposes to secondary bacterial pneumonia is poorly understood, but research suggests that viral-mediated alterations in bactericidal ability of macrophages, decreased bacterial clearance, and immunosuppression are important.

PIV3 infects ciliated respiratory epithelium of the upper and lower airway, as well as alveolar epithelium and macrophages.[10, 31, 50] Ciliated epithelium is important as a mechanical barrier to bacterial colonization and is important in removing bacteria and other particulates from the upper airway. Alveolar macrophages serve the function of bacterial clearance in the alveoli by phagocytosis and killing of bacteria. Therefore, PIV3-mediated lysis or altered function of respiratory epithelium and alveolar macrophages facilitate pulmonary bacterial colonization. PIV3 infects bovine alveolar macrophages in vitro and in vivo.[32, 49]

Macrophages support PIV3 replication, resulting in cell death or altered function. Macrophages collected from calves and infected with PIV3 in vitro have decreased phagocytosis and killing of bacteria.[18, 28, 29, 32] Infected macrophages have altered fatty acid metabolism and secretion, resulting in release of immunosuppressive prostaglandins.[29] Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin synthesis partially reverse depressed bactericidal functions.[28] These experimental findings suggest that NSAIDs may restore viral damaged host defense mechanisms, thereby justifying their use in bovine respiratory disease.

Alveolar macrophages modulate pulmonary inflammation and immune responses that are altered by PIV3 infection. Peripheral blood mononuclear cells isolated from PIV3-infected calves have decreased in vitro proliferative responses to lymphocyte mitogens.[40] The mechanism of immunosuppression caused by PIV3 is poorly understood, but infection of macrophages and monocytes may contribute to decreased lymphocyte functions. Bovine alveolar macrophages and monocytes can serve as accessory cells for antigen- and mitogen-stimulated lymphocyte proliferation.[2, 4] Infection of macrophages with PIV3 results in a significant decrease in their ability to support lymphocyte proliferation.[4] Even though PIV3-infected macrophages secrete potentially immunosuppressive factors, it was shown experimentally that PIV3-induced depression of lymphocyte proliferation was not due to secreted soluble factors, but required direct contact with activated lymphocytes. In vitro studies concluded that close contact between PIV3-infected alveolar macrophages and stimulated lymphocytes facilitated viral infection of lymphocytes.[5] Lymphocytes had intracytoplasmic viral inclusions similar to those seen in macrophages and respiratory epithelium. These studies were the first to show PIV3-infected lymphocytes and that infection resulted in altered lymphocyte functions.[5]

Infection of calves with PIV3 is rarely fatal, producing mild or inapparent clinical signs. PIV3 is important in mixed infections, and may predispose to bacterial pneumonia by altering bacterial clearance in the upper airway and lung. Immunosuppression may result from altered macrophage and lymphocyte functions as the result of virus infection of these cells and release of immunosuppressive factors.

Properties of PI-3 Virus

Parainfluenza viruses are pleomorphic, and their average diameter ranges from 150 to 200 nm. These viruses have a lipid bilayer membrane with spike-like surface projections, such as hemagglutinin-neuraminidase (HN) and fusion (F) proteins. These surface proteins impart hemadsorption ability (binding of erythrocytes to infected cells), hemagglutinating, and hemolytic (lysis of adsorbed erythrocytes) abilities to the virus. Most of the PI-3 virions contain a negative-stranded genome, and

a small number of virions contain positive-sense, replicative intermediate RNA (antigenome).

Epidemiology

PI-3 virus infection occurs worldwide, and is usually subclinical. Clinical disease is precipitated by the stress of adverse environmental conditions. Following natural infection, PI-3 virus may persist for several weeks in infected animals. Various factors, such as immunity and environment, affect the transmission of PI-3 virus. The disease is seen commonly in calves between 2 to 8 months of age. Maternal immunity wanes at about 2 months, and these calves become susceptible to PI-3 infection. The infection is common in calves raised under intensified conditions. Aerosol infection and direct contact are the methods of transmission, and both are accentuated in crowded, inadequately ventilated conditions.

Pathogenesis

Clinical Signs

Like all viral pneumonias of calves, the clinical signs of PI-3 infection include a febrile reaction (104–105°F) at about the 5th day, followed by rhinitis and pneumonia. A harsh, hacking cough is stimulated easily by pinching the trachea. In field cases the clinical findings are similar, but fever may be higher. Most infections with PI-3 are mild with coughing, nasal discharge, slight fever, and recovery in a few days.

Immunity

Antigenic differences between the human and bovine PI-3 viruses have been described.[26] Monoclonal antibodies have revealed extensive antigenic differences between the HN glycoproteins of human and bovine PI-3 viruses,[41] and monoclonal antibodies have been used to show antigenic differences between PI-3 strains within a species.[44] Immunities to both the HN and F surface antigens play a major role in host resistance.[35, 36] Anti-H antibody inhibited attachment of the virus to the host cell whereas anti-F antibody inhibited both viral penetration of the host cell and cell-to-cell spread of infection by recruitment of uninfected cells through cell fusion. These findings are consistent with the known function of the individual surface glycoproteins. For example, passive immunization with monoclonal antibody against the fusion domain of the F protein or the hemagglutinin or neuraminidase domain of the HN protein protected mice against challenge with Sendai virus, a paramyxovirus.

The role of different glycoproteins of PI-3 in protection has been

examined.[47] Mice immunized with recombinant vaccinia carrying -HN or -F reduced PI-3 replication by more than 3000-fold and 6-fold, respectively. Although vaccinia-expressed HN induced only a threefold greater level of neutralizing antibodies than did vaccinia-expressed F protein, it conferred a 500-fold greater level of protection in the upper respiratory tract. This dissociation between the level of neutralizing antibodies and protection suggested that factors other than circulating neutralizing antibodies are important in host resistance to PI-3.

In uncomplicated PIV3 infections, gross lesions and clinical signs are most evident 4 to 12 days following experimental or natural infection. Mild mucopurulent inflammation of nasal passages and upper airway manifest as serous to mucopurulent nasal discharge. Nasal mucosa may be hyperemic. Early lung lesions are bilateral, red to purple foci of parenchymal collapse and consolidation limited to the cranioventral lung lobes. Coalescing foci progress to more extensive parenchymal collapse unless secondary bacterial involvement occurs that results in severe fibrosuppperative bronchopneumonia.

PIV3 infects a variety of cell types in the upper airway, as well as cells of the alveoli. Initial viral replication in ciliated, nonciliated, and mucous epithelium of the upper airway results in acute necrotizing bronchitis and bronchiolitis. Extension into alveoli where type II pneumonocytes and macrophages are infected leads to filling of alveoli with exudate-containing macrophages, neutrophils, lymphocytes, and fibrin with extravasated erythrocytes and edema fluid. Parenchymal collapse results from inflammation and obstruction of alveoli and bronchioles with inflammatory exudate.

Acidophilic cytoplasmic inclusions are most often seen 2 to 4 days postinfection in degenerate bronchial epithelium, and less so in bronchiolar and alveolar lining cells. Multinucleated alveolar lining cells with cytoplasmic viral inclusions can occur, but are uncommon. Inclusions are less common in calves that die of respiratory disease because they are absent or obscured late in the infection. In natural infections, viral-mediated damage and replication is more limited to airways than alveoli, resulting in bronchiolitis rather than pneumonia, versus experimental infections, where high doses of virus are administered to alveoli. Bronchiolar epithelial proliferation and fibrosis can result in bronchiolitis fibrosa obliterans accompanied by alveolar type II epithelial hyperplasia. Infiltrates of lymphocytes and plasma cells accumulate in the peribronchial and perivascular interstitium.

Laboratory Diagnosis

Definitive diagnosis of PIV3 requires recovery of the virus from respiratory secretions and tissues. Hemadsorbing viruses, such as PIV3, traditionally have been diagnosed by isolation in cell culture followed by hemadsorption with guinea pig erythrocytes.[37] PI-3 virus can be isolated in bovine turbinate and bovine kidney cell cultures; however,

the cell culture needs to be incubated at 35°C. Lung tissue from an infected animal is the specimen of choice. For quick diagnosis the PIV3 antigen can be identified in respiratory specimens by immunofluorescence or immunoperoxidase tests. Serologic tests (hemagglutination-inhibition or viral neutralization) are useful in supporting the diagnosis of respiratory infection with PIV3 virus. Because PIV3 is ubiquitous in cattle populations, the virus isolation and serologic tests are not necessarily diagnostic of PIV3 as a cause of the disease. Antibodies to the HN glycoprotein are measured by hemagglutination and neuraminidase inhibition. Further, antihemagglutinin antibodies can be quantified by a plaque reduction neutralization test. Antibodies to viral fusion protein can be measured by immunoprecipitation or by inhibition of syncytia formation in infected cell cultures.

BRCV

Role of BRCV in BRD

Coronaviruses are a common cause of upper respiratory tract disease in calves[17] and people[51]; however, information on the role of bovine coronavirus in lower respiratory tract disease is limited. All four of Koch's postulates to associate BRCV with upper and lower respiratory tract disease have been fulfilled.[21] There are a few reports of detection of BRCV antigen in the lungs.[22] A combination of factors may predispose the animals to clinical disease, as BRCV has not been commonly associated with clinical disease in nature.

Properties of BRCV

BRCV is a pleomorphic, positive-stranded RNA virus with spike-shaped projections on the surface.[30] It has two surface glycoproteins, spike, and hemagglutinin-esterase.[13] Small membrane protein (sM) recently has also been recognized as a surface protein. These surface glycoproteins are embedded in the membrane protein (M). An internal phosphoprotein, the nucleocapsid protein, is wrapped around the genomic RNA. Due to the presence of a lipid envelope, coronaviruses are very susceptible to heat, lipid solvents, and disinfectants. Like all coronaviruses, BRCV makes a nested set of mRNAs (n = 9) that have common sequence at the 3' end. Only unique regions of the transcripts are translated. Each mRNA also has a leader sequence at the 5' end.[30]

Epidemiology

BRCV infections have been reported after shipment and commingling of adult cattle purchased from various sources.[48] In a retrospective

study on clinical submissions from Wisconsin and Kansas during 1994 to 1996, BRCV antigen was not detected in lungs of cattle with a history of respiratory disease; however, respiratory disease was experimentally reproduced after oral inoculation with a BRCV isolate in colostrum-deprived calves (Minnesota-1).[22] Other factors, such as lower environmental temperature, that increase the survival of the virus in the environment of cattle, and the immune status of the calves may play an important role in the transmission of the virus. Chronically infected adult cattle may be the major source of the virus; however, there is no published report that BRCV persists in the respiratory tract after infection. Several factors, such as passively transferred antibodies in colostrum, ambient temperature, and strain of BRCV, may play a role in determining the outcome of the disease. Epidemiologic patterns provide some evidence of differences in tissue tropism among different BRCV isolates. About 50% of the infections in calves have both enteric and respiratory tract involvement; however, only the respiratory tract (nostril, trachea, or lungs) may be involved in 25%, and only the enteric cavity is involved in the other 25% of the cases.

Chronic BRCV Infections

BCV is known to establish chronic infections in the intestinal tract of calves and adult cattle. During chronic infection the virus is excreted intermittently at low titers in the feces, and can contaminate the environment.[24] Most isolates of BCV produce both enteric and respiratory infections. After the acute phase of the BCV infection that lasts for about 3 days, the virus is detectable only at low titers in the feces. Even in adult cattle, the virus is excreted in feces when the animals are stressed, such as after parturition.[14] Tissues and cell types in which BCV persists in vivo are not known; however, mesenteric lymph nodes and respiratory tissues may be the targets of chronic BCV infection. The molecular mechanism of chronic BCV infection in vivo has not been studied.

Pathogenesis

Clinical Signs

Experimentally, signs of respiratory disease develop 3 to 4 days after inoculation of pneumotropic isolates of BCV.[22] Under experimental conditions, neonatal, colostrum-deprived calves develop respiratory distress, such as wheezing and open-mouth breathing. Under natural conditions respiratory disease caused by BRCV can be observed in calves aged 6 to 9 months, and these calves developed fever, nasal discharge, and respiratory distress.[48]

Immunity

After natural and experimental infections with BRCV, the antibodies are produced against all the structural proteins of BCV, nucleocapsid (N), membrane protein (M), peplomer protein (S), and hemagglutinin-esterase (HE) proteins. The active antibody responses in the intestinal and respiratory tracts to BCV are associated with all the classes of immunoglobulins, but the sequence of induction of different isotypes is different.[25] Presence of passive colostral antibodies may influence the development of active immunity at the mucosal surface; however, the presence of passive antibodies may decrease the severity of the clinical signs and prevent mortality after a severe infection. Which class of antibodies is involved in protection against BCV infection has not been established experimentally.

Gross Lesions

On experimental infection with a pneumoenteric isolate of BRCV, neonatal, colostrum-deprived calves develop congestion, hemorrhage, and edema of the interlobular septa. The ventrolateral areas of the lungs are mainly involved.[21]

Microscopic Lesions

Based on experimental infections, calves with BRCV-associated respiratory disease develop interstitial pneumonia with the presence of erythrocytes in the alveoli and congestion of blood vessels. An important histologic feature of BRCV is thickening of alveolar septa by infiltration of mononuclear inflammatory cells in the interstitial areas of the lungs.[21]

Laboratory Diagnosis

In a living calf, nasal swabs collected during the acute stage of upper respiratory tract disease are the specimens of choice for the diagnosis of BRCV.[21] The swabs can be submitted in saline with broad-spectrum antibiotics to a diagnostic laboratory for a direct fluorescent antibody test.[22] Nasal swabs are easy to collect, and yield a large number of nasal epithelial cells. On receiving the nasal swabs in the diagnostic laboratory, the nasal cells should be washed thoroughly with phosphate-buffer saline (PBS) (0.01M, pH 7.2) and spotted on Teflon-coated well slides for examination by the direct fluorescent antibody test. Often, BRCV antigen-positive cells appear pear shaped or they may fuse to form syncytia.[21] The specific apple-green fluorescence is localized in the cytoplasm of the antigen-positive cells. BCV is difficult to isolate in cell culture[23]; however, there are a few reports of successful isolation of BRCV in human rectal tumor-18 cell line.[48] Examination of nasal specimens by direct electron microscopy is difficult to interpret because

coronavirus particles lose their surface projections (peplomers) during specimen preparation.[17] Serology is helpful and supportive of the diagnosis of BRCV, but antigen detection tests are preferred because almost all adult cattle are serologically positive for BRCV antibodies.

Vaccination and Treatment

Enteric and respiratory isolates of BCV are related antigenically.[56] Thus, an effective vaccine developed for enteric BRCV also may protect against the respiratory disease. The severity of respiratory BRCV infection can be reduced by feeding colostrum to newborn calves. In cell culture, hygromycin B inhibits BCV transcription and protein production.[57] Hygromycin B has no known toxicity in cattle after long-term feeding under feedlot conditions. Because hygromycin B has been found to inhibit mouse hepatitis virus infection in vitro and in vivo, it is hoped hygromycin B will prove to be a suitable treatment for BCV-associated infections in calves.

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Address reprint requests to

Sanjay Kapil, DVM, PhD
Diagnostic Medicine
College of Veterinary Medicine
1800 Denison Avenue
Manhattan, KS 66506