Bovine Respiratory Coronavirus

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- Wild ruminant coronaviruses

Bovine coronaviruses (BCoVs) cause respiratory and enteric infections in cattle and wild ruminants.[1–3] BCoVs belong to the family Coronaviridae in the order Nidovirales and are members of subgroup 2a along with swine hemagglutinating encephalomyelitis virus (HEV), canine respiratory CoV, and human CoV-OC43 and HKU1. HEV, which causes wasting disease, is an exception;[4] the others cause enteric and/or respiratory disease. Recently discovered severe acute respiratory syndrome (SARS)-CoVs, which are associated with respiratory and enteric infections in humans and animals (eg, civet cats, raccoon dogs, bats), belong to a new CoV subgroup 2b.[1,5]

BCoV is a pneumoenteric virus that infects the upper and lower respiratory tract and intestine. BCoV is shed in feces and nasal secretions and infects the lung. BCoV is the cause of 3 distinct clinical syndromes in cattle: calf diarrhea (CD),[1,2] winter dysentery (WD) with hemorrhagic diarrhea in adults,[3,6–12] and respiratory infections in cattle of various ages including the bovine respiratory disease complex (BRDC) or shipping fever of feedlot cattle.[2,13–25] All BCoV isolates examined to date, regardless of clinical origin belong to a single serotype based on virus cross-neutralization tests.[8,14] Although 2 to 3 subtypes of BCoV as determined by biologic properties and antigenic variation identified by neutralization tests or using monoclonal antibodies (MAbs) are recognized, each encompasses viruses from all 3 clinical syndromes.[2,3,8,14,15] Despite genetic differences (point mutations but not deletions) detected in the spike (S) gene between enteric and respiratory isolates, including ones from the same animal,[26–28] in vivo challenge revealed a high level of cross-protection between such isolates.[29,30] No consistent antigenic or genetic markers have been identified to discriminate BCoVs from the different clinical syndromes. Reviews describing the role of BCoV in CD and WD are available.[1,3,7] This article focuses on respiratory BCoV infections including

viral characteristics, epidemiology and interspecies transmission, diagnosis, pathogenesis and clinical signs, and immunity and vaccines.

VIRAL CHARACTERISTICS

BCoV is enveloped and pleomorphic, ranging from 65 to 210 nm in diameter, and covered with a double layer of short (hemagglutinin) and long (spike) surface projections (**Fig. 1**).[2] Like other enveloped viruses, BCoV is sensitive to detergents and lipid solvents (eg, ether, chloroform) and is inactivated by conventional disinfectants, formalin, and heat. The large genome consists of single-stranded, positive-sense RNA of 27 to 32 kb encoding 5 major structural proteins. Among these, the 50-kDa nucleocapsid (N) is highly conserved among strains, so it is often the target for viral RNA detection assays.[29] Unique to some group 2 CoVs including BCoV and wild ruminant CoVs, is the presence of a surface hemagglutinin-esterase (HE) glycoprotein (120–140 kDa). The HE acts as a receptor-destroying enzyme (esterase) to reverse hemagglutination. Like other CoVs, BCoV also possesses an outer-surface S glycoprotein (190 kDa). It consists of an S1 subunit that contains the dominant neutralizing epitopes and an S2 subunit that mediates viral membrane fusion. HE and S are important viral proteins that are involved in attachment to host cell receptors and hemagglutination of chicken, rat, mouse, and hamster erythrocytes. MAbs to the HE or S protein prevented BCoV-induced villous atrophy in vivo in intestinal loops of calves, confirming their dual role in in vivo protection.[31] HE and S proteins elicit neutralizing antibodies that can block viral attachment and infectivity, so they are important for immunity and vaccines.

Variation in tissue tropism and host range among CoVs is attributed mainly to changes in the S protein. Researchers have sequenced the partial or full-length S gene of multiple BCoV strains to ascertain the genetic basis of the broad host range of BCoV (see section on Epidemiology) and occurrence of the distinct clinical syndromes. Several groups have compared the S (or S1) or full-length genomic sequences[22,26–28,32–36] of WD or respiratory and enteric BCoV isolates, including isolates from the same animal. The porcine respiratory CoV evolved as an S gene deletion mutant (deletions of 621–681nuceotides) of swine transmissible gastroenteritis virus, acquiring an almost exclusive respiratory tropism.[37] No similar large S gene deletions were detected in respiratory BCoV strains, most of which also possess an
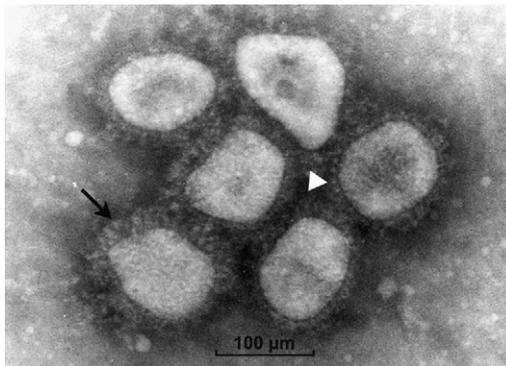

Fig. 1. Immune electron microscopy of tissue culture-adapted respiratory BCoV. Particles reacted with antisera to BCoV showing shorter surface HE (*arrowhead*) and longer spikes (*arrow*) resulting in a dense outer fringe. Bar equals 100 μm.

enteric tropism as revealed by calf challenge studies.[29] Focusing on the hypervariable region (amino acids [aa] 452–593) containing the neutralizing epitope (S1B) of the S1 subunit, 4 groups[22,26,27,36] reported that respiratory strains (or respiratory and enteric isolates from the same feedlot calf) had changes in aa residues 510 and 531 compared with the reference enteric Mebus strain and a WD strain (DBA). One of the polymorphic positions (aa 531) discriminated between enteric (aspartic acid or asparagine) and respiratory (glycine) BCoV strains in 2 studies,[26,36] but not in others.[27,28,34] Therefore, like the antigenic and biologic differences observed among BCoV isolates, variability was not necessarily related to the clinical origin of the isolates.[14,15] BCoV, like other RNA viruses, represents a quasispecies or swarm of viruses;[38] some viruses within a population may be more suitable for replication in respiratory versus intestinal sites, contributing to the sequence differences reported for paired enteric/respiratory isolates from the same host.[28] Zhang and colleagues[28] noted that in the process of cell culture adaptation, an enteric BCoV strain accumulated mutations to resemble the corresponding respiratory BCoV isolate from the same animal. Consequently interpretation of the comparative sequence analysis of enteric and respiratory strains of BCoV may be compromised by lack of complete genome sequences and the laboratory manipulation of field strains (multiple cell culture passage and plaque isolations) before sequencing.

All investigators showed that the greatest aa sequence divergence (42 aa changes at 38 distinct sites)[27] and the most differences by phylogenetic analysis were between the historic reference Mebus enteric BCoV strain (1972) and the more recent BCoV isolates, regardless of their clinical origin. This result demonstrates that BCoVs, like other RNA viruses, are evolving in the field, with recent isolates more similar to contemporary strains than to historic strains. If the genetic divergence affects neutralizing or other key epitopes allowing viruses to escape existing immunity,[36] then vaccines containing more contemporary or broadly cross-reactive BCoV strains may be required to enhance the efficacy of existing or newly developed BCoV vaccines. Sequential sera from cattle with respiratory BCoV infections associated with BRDC pneumonia reacted with higher neutralizing and hemagglutination inhibition (HI) titers to a more contemporary respiratory lung isolate (97TXSF-Lu15-2) than to much older historic enteric BCoV strains, including a derivative of the high cell culture–passaged Mebus 1972 strain (L9-81) and an older wild type strain (LY138-3).[39] However, in a previous report from the same laboratory,[35] an earlier isolate of a respiratory BCoV strain (BRCV-G95) was more similar to the enteric LY-138 strain than to the older Mebus strain L9-81 based on S-specific MAb reactivity. These data concur with the greater phylogenetic and antigenic divergence described between the Mebus cell culture–passaged strain compared with the more recent enteric and respiratory BCoV isolates.[14,15,28,40] In the studies by Lin and colleagues,[39] it is likely that the antigenic variation observed reflects the timeframe differences, independently of the enteric versus respiratory origin of the isolates. Repeating these immunologic assays using more recent enteric BCoV strains is necessary to clarify this issue.

EPIDEMIOLOGY AND INTERSPECIES TRANSMISSION
Respiratory BCoV Infections in Calf Respiratory Disease

BCoV is ubiquitous in cattle worldwide based on BCoV antibody seroprevalence data.[2,3,7] Respiratory BCoV is associated with mild respiratory disease (coughing, rhinitis) or pneumonia in 2- to 6-month-old calves. It is detected in nasal secretions, lung, and often intestine as well as feces (**Table 1**).[2,3,16,24,41–44] Four respiratory

Table 1
Summary of clinical signs and pathogenesis of respiratory BCoV infections

| Disease Syndrome | Clinical Signs | Cells Infected | Lesions | | Shedding[a] | | Ages Affected |
			Respiratory	Enteric	Nasal	Fecal	
Calf pneumonia	Cough Rhinitis ± Pneumonia ± Diarrhea Fever/anorexia	Nasal/ ± lung Tracheal ± Intestinal Epithelial cells	± Pneumonia	± J, I, Colon Villous atrophy	Sporadic 5 d	Sporadic 5 d	2 wk–6 mo
Shipping fever/BRDC	Cough/dyspnea ± Rhinitis ± Pneumonia ± Diarrhea Fever/anorexia	Nasal/tracheal Bronchi/alveoli ± Intestinal Epithelial cells	Interstitial Emphysema Bronchiolitis Alveolitis	± NT	5–10 d (17 d)	4–8 d (17 d)	6 mo–10 mo

In experimental challenge studies, the incubation periods for disease onset and shedding ranged from 3 to 8 days.

Abbreviations: I, ileum; J, jejunum; NT, not tested.

[a] Shedding detected by infectivity or antigen assays; data in parentheses denote shedding detected by reverse transcriptase polymerase chain reaction.

disease outbreaks associated with BCoV infection but without concurrent detection of other common respiratory viruses, bacteria, and mycoplasmas were investigated in dairy and beef cattle in Italy.[41] In 3 of 4 outbreaks, the classic signs of bovine respiratory disease (dyspnea, fever, respiratory distress) were evident in 2- to 3-month-old calves, and BCoV was detected by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in nasal samples. In 2 of 4 outbreaks, respiratory disease and enteric disease were present: BCoV was detected simultaneously in nasal and fecal specimens. In 1 of these 2 herds, clinical signs were present in calves, heifers, and lactating dairy cattle. In the second, concurrent infection with a group A bovine rotavirus was detected. Researchers also detected ocular shedding of BCoV in lower titers in one of the herds, suggesting that ocular swabs could be another diagnostic specimen for detection of BCoV. BCoV was isolated in human rectal tumor (HRT)-18 cells from nasal swabs of calves from 3 of the 4 outbreaks.

BCoV seroprevalence studies in 135 Norwegian dairy herds indicated that calves (>150 days old) in herds with BCoV-seropositive calves had an increased risk (hazard ratio = 3.9) of respiratory disease compared with herds with BCoV-seronegative calves.[45] In studies of dairy calves in Ohio from birth to 20 weeks of age, Heckert and colleagues[16,24] documented fecal and nasal shedding of respiratory BCoV, but with diarrhea prominent in the initial infection. Subsequently, repeated or intermittent nasal shedding episodes occurred in the same animal, with or without respiratory disease, but with transient increases in serum antibody titers consistent with reinfection. These findings suggest a lack of long-term mucosal immunity in the upper respiratory tract after natural respiratory BCoV infection, confirming similar observations for human[46] and porcine respiratory CoV infections.[47]

Within a herd, reservoirs for respiratory BCoV infection may be virus cycling in clinically or subclinically infected calves, young adult cattle in which sporadic nasal shedding occurs,[16,24] or clinically or subclinically infected adults.[3,48] Respiratory BCoV is transmitted via fecal-oral and potentially respiratory (aerosol) routes.

Respiratory BCoV Infections Associated with the BRDC in Feedlot Cattle

Respiratory BCoV has been increasingly implicated since 1995 in BRDC associated with respiratory disease and reduced growth performance in feedlot cattle (see **Table 1**).[13,17–21,23,25] Respiratory BCoV was detected from nasal secretions and lungs of cattle with pneumonia and/or from feces.[15,17–21,23,25] In a follow-up study, a high percentage of feedlot cattle (45%) shed BCoV nasally and in feces as determined by enzyme-linked immunosorbent assay (ELISA).[13] Application of nested RT-PCR detected higher respiratory BCoV nasal and fecal shedding rates of 84% and 96%, respectively.[23] Storz and colleagues[20] reported that 25 of 26 Texas feedlot cattle that died had a respiratory BCoV infection. Respiratory BCoV and *Pasteurella* sp were isolated from lungs of the cattle with necrotizing pneumonia.

Some investigators have shown an association between nasal shedding of respiratory BCoV and respiratory disease. In a large feedlot study (n = 1074 cattle), Lathrop and colleagues[17] noted that feedlot calves shedding respiratory BCoV nasally and seroconverting to respiratory BCoV (>4-fold) were 1.6 times more likely to have respiratory disease and 2.2 times more likely to have pulmonary lesions at slaughter than animals that did not shed respiratory BCoV. Similarly, in studies by Hasoksuz and colleagues[23] and Thomas and colleagues,[25] calves shedding respiratory BCoV nasally were 2.7 and 1.5 times, respectively, more likely to have respiratory disease than calves that were not shedding. Nasal shedding of respiratory BCoV or an antibody titer less than 20 increased the risk of requiring treatment for respiratory disease in another report.[49] Intranasal (IN) vaccination of such calves using a commercial, modified, live

BCoV calf vaccine on entry to a feedlot reduced the risk of treatment for BRDC in the latter study.

Respiratory and enteric shedding of respiratory BCoV are common in feedlot cattle with peak shedding at 0 to 4 days after arrival at feedlots. In one study, 3-day pre-arrival specimens were tested, and nasal shedding consistently preceded fecal shedding.[25] Many cattle (61%–74%) shed respiratory BCoV at the buyer-order before shipping to feedlots.[21] A high percentage (91%–95%) of feedlot cattle seroconverted (2–4-fold increased titers) to respiratory BCoV by 3 weeks after arrival.

An important observation from several studies was that cattle arriving with relatively high respiratory BCoV antibody ELISA titers or neutralizing antibodies in serum were less likely to shed respiratory BCoV, seroconvert, or develop BRDC.[13,18,25,50,51] Furthermore, some investigators showed that respiratory BCoV infections had a negative effect on weight gains in feedlot cattle,[13,23] which suggests that respiratory BCoV may affect herd health and performance. Conversely, higher serum antibody titers against respiratory BCoV have been associated with increased weight gains.[25,50]

BCoV Interspecies Transmission and Wildlife Reservoirs

Possible wildlife reservoirs for BCoV have been identified. Captive wild ruminants from the United States including sambar deer (*Cervus unicolor*), white-tailed deer (*Odocoileus virginianus*), waterbuck (*Kobus ellipsiprymnus*), elk (*Cervus elephus*), and more recently, giraffe[52] harbor CoVs that are closely related to BCoV biologically, genetically, and antigenically (cross-neutralizing).[53,54] Bovine-like CoVs have also been identified in water buffalo calves[55] and in alpacas.[56] Deer and waterbuck isolates were from animals with bloody diarrhea resembling WD in cattle. Serologically, 6.6% and 8.7% of sera from white-tailed deer in Ohio and mule deer in Wyoming, respectively, were seropositive for antibodies to BCoV by indirect immunofluorescence tests.[54] Caribous (*Rangifer tarandus*) were also BCoV seropositive.[57] These studies confirm the circulation of CoVs that are antigenically closely related to BCoV in captive and native wild ruminants.

Several CoVs from wild ruminants have recently been fully sequenced to assess their genetic similarity to BCoV.[40,52] The antigenically related giraffe, sambar and white-tailed deer, waterbuck, and sable antelope CoVs share high (99.3%–99.6%) aa sequence identity with enteric and respiratory BCoV strains, supporting their close genetic relatedness. Wild ruminant CoV isolates from sambar and white-tailed deer and waterbuck also infected the upper respiratory and intestinal tracts of gnotobiotic calves and caused diarrhea,[54] affirming experimentally that wild ruminants may serve as a reservoir for CoV strains that are transmissible to calves. The possibility exists that native wild ruminants may transmit bovine-like CoVs to cattle (see later) or vice versa. Few serologic surveys of wild ruminants in native habitats have been done. A particular concern is that such interspecies infections may culminate in more genetically divergent BCoV strains including recombinant strains, increasing the possibility of their transmission to other species. Analysis of CoV genomic sequence data has revealed that porcine HEV and human respiratory CoV OC43 represent CoVs that likely evolved from ancestral BCoV strains.[58]

Many CoVs have restricted host ranges; some such as the BCoV and more recently the SARS-CoV seem to be promiscuous.[5,59] Coronaviruses genetically (>95% nucleotide identity) and/or antigenically similar to BCoV have been detected from respiratory samples of dogs with respiratory disease,[60] and from humans[61] and wild ruminants (see earlier discussion). A human enteric CoV isolate from a child with acute diarrhea (HECoV-4408) was genetically (99% nucleotide identity in the S and HE gene with BCoV) and antigenically closely related to BCoV, suggesting that it is a BCoV

variant able to infect humans. A recent report confirmed that the HECoV-4408 strain infects (upper respiratory tract and intestine) and causes diarrhea and intestinal lesions in gnotobiotic calves. This strain induced complete cross-protective immunity against the virulent BCoV-DB2 enteric strain. An enteric BCoV also experimentally infected dogs, causing subclinical infection and seroconversion.[62] Furthermore, the virulent BCoV-DB2 enteric strain caused mild disease (diarrhea) in phylogenetically diverse species such as avian hosts, including baby turkeys but not baby chicks.[63] These data raise intriguing questions of whether dogs or wild birds (such as wild turkeys) can also be a reservoir for bovine-like CoVs transmissible to cattle or wild ruminants, or conversely, if cattle (or ruminants) can transmit CoVs to dogs, wild birds, or poultry. There are few if any seroprevalence surveys for bovine-like CoVs in avian species and only limited data for wild ruminants.[54,57] The reasons for the broad host range of BCoV are unknown but may relate to the presence of a hemagglutinin on BCoV and its possible role in binding to diverse cell types. Experimental evidence for interspecies transmission of bovine-like CoVs between wild ruminants, dogs, birds, and cattle is of concern for open feedlots where wild birds may congregate or cattle may be exposed to dogs, wild ruminants, or their feces.

DIAGNOSIS

BCoV commonly infects the upper respiratory and the intestinal tract with shedding detected in nasal secretions and/or feces.[13,23,43,64] BCoV has also been detected or isolated from lung in animals with respiratory disease. Uncomplicated BCoV infections are characterized as acute infections with a short duration of virus shedding (see **Table 1**). The sensitivity of the assay used to detect BCoV shedding influences the detection rates and the length of time that the virus is detected. Generally, nucleic acid detection assays are among the most sensitive (RT-PCR, nested RT-PCR, real-time qRT-PCR) barring interference by inhibitors common in fecal samples.

The acute transient nature of BCoV infections necessitates sample collection at disease onset or shortly thereafter (see **Table 1**). For BRDC-associated infection, the peak of BCoV nasal (or fecal) shedding is often seen on entry into the feedlot or within the first week of arrival.[13,21,23,25] This pattern of BCoV shedding is likely attributable to the stress of shipping and the transmission of new BCoV strains among mixed source, comingled calves from auction barns.[21,25] For cattle with BRDC, differential diagnosis is needed because multiple respiratory viral and bacterial pathogens contribute to this syndrome and the ensuing fatalities.

To diagnose BCoV from animals with suspected respiratory (or enteric) disease necropsy, samples to be submitted include tissues from the upper respiratory tract (eg, nasal, pharyngeal, tracheal tissues) and lung,[14,20,21] and as available tissues from the distal small intestine and colon.[64] For live animals, ideally, nasal secretions collected using sterile polyester-tipped swabs (1 per nostril) and feces collected in sterile fecal cups (via anal stimulation using sterile gloves) should be transported on ice to the diagnostic lab.[13,15] Also tracheobronchial lavage fluids can be aspirated from live calves with acute respiratory disease. In at least 1 study, they were found to be positive for BCoV antigen by ELISA.[65]

BCoV infections can be diagnosed by detection of virus, viral antigen, or viral RNA in tissues, secretions, or excretions of infected animals. Comprehensive methods used by our laboratory for the isolation and detection of BCoV from nasal secretions, lung homogenates or feces, or titration of BCoV serum or neutralizing antibodies have been published recently[66] and are not reiterated in detail in this article. These methods

include procedures for primary virus isolation, propagation, and plaque induction in our cloned line of HRT-18 cell cultures.[15,20,21,23] This cell line is used most successfully and almost universally for isolation of respiratory and enteric BCoV strains and bovine-like CoVs from wild ruminants and dogs. Some BCoV field strains fail to grow in cell culture, so sensitivity for BCoV detection may be low.

For viral antigen detection in respiratory (trachea, lung) or intestinal (ileum, colon) tissues (frozen or paraffin-embedded), immunofluorescent or immunohistochemical staining is performed using hyperimmune antiserum or MAbs to BCoV. Detection of BCoV in nasal secretions or feces is accomplished by immune electron microscopy, which has the advantage of detecting other viruses, but its sensitivity is relatively low.[16,54] BCoV antigens are most commonly detected by ELISA using MAbs to improve assay specificity and sensitivity. Advantages include rapid test results and applicability to large sample numbers. Highly sensitive molecular assays to detect BCoV RNA in nasal secretions, lung lysates, or feces are becoming more widely used. These assays include RT-PCR and the more sensitive nested RT-PCR and real-time qPCR assays.[29,67] Ideally these assays should target conserved regions of the BCoV genome (polymerase or N protein) to detect divergent strains. For feces, controls are needed to detect interference by PCR inhibitors that need to be eliminated or diluted out for assay validity.

Antibodies to BCoV can be quantitated by virus neutralization and HI tests that measure functional neutralizing or hemagglutinating antibodies, respectively, that often correlate with immunity.[14,51] In addition, ELISAs are used to quantitate overall or isotype-specific antibodies (IgM, IgA, IgG1, IgG2) in serum, nasal secretions, or feces, because certain isotypes (ie, IgA, IgG1, IgG2) may be better correlated with mucosal immunity or neutralizing or HI antibodies.[16,24,51,68,69] Because BCoV antibodies are widespread in cattle, paired acute and convalescent serum samples are needed for serologic diagnosis of BCoV infections.

PATHOGENESIS AND CLINICAL SIGNS

Research on respiratory and enteric BCoV infections of cattle has provided important information on BCoV disease pathogenesis, possible potentiators for increased disease severity, and vaccine strategies. Enteric BCoV infections are generally most severe in calves. Respiratory BCoV infections in adults are more severe or even fatal when combined with other factors including stress and transport of animals (shipping fever of cattle) that increase corticosteroid levels, high doses of virus exposure, aerosols, and coinfections with other respiratory pathogens (viruses, bacteria, bacterial lipopolysaccharides). Such variables, by accentuating viral shedding or increasing titers shed may also contribute to virus transmission.

Respiratory BCoV Pathogenesis in Younger Calves and Cattle with WD

Besides causing diarrhea in young calves (1–3 weeks of age) and cows with WD, BCoV is implicated as a cause of mild respiratory disease (coughing, rhinitis) or pneumonia in 2- to 6-month-old calves.[16,24,42–44] Clinical signs include coughing, fever, rhinitis, and inappetence, often with concurrent diarrhea. BCoV has been isolated from nasal and pharyngeal swabs and lung wash of sick calves. Experimental calf challenge studies using calf respiratory BCoV isolates confirmed fecal and nasal shedding (averaging 5 days) and diarrhea, but only variable mild respiratory disease.[42,43] BCoV respiratory infections in the field are likely exacerbated by stress or respiratory coinfections that may include the common bovine respiratory viruses, bacteria, and Mycoplasma sp.

In 2 experimental studies of WD in BCoV-seronegative or -seropositive dairy cows, fecal shedding of BCoV was coincident with or preceded diarrhea and persisted for 1 to 4 days. No respiratory disease or fever was evident in the BCoV-seropositive cows, but nasal shedding of BCoV was detected in 1 of 5 cows.[6] BCoV-seronegative cows directly exposed to calves experimentally infected with a WD strain of BCoV developed transient fevers, mild cough, and serous mucopurulent discharge.[10] These data are consistent with field outbreak reports indicating variable signs of respiratory disease in cattle with WD.[7,9] WD isolates of BCoV are antigenically closely related to calf enteric and respiratory isolates, but the various strains, regardless of clinical origin, elicited cross-protection against one another in calf challenge studies.[12,29,30]

A plausible scenario for the pathogenesis of BCoV and its transit to the intestine has been proposed based on the time course of BCoV nasal and fecal shedding in natural and experimental BCoV infections.[16,25,64] Following initial and extensive replication in the nasal mucosa, BCoV may spread to the gastrointestinal tract after the swallowing of large quantities of virus coated in mucous secretions. This initial respiratory amplification of BCoV and its protective coating by mucus may allow larger amounts of this labile, enveloped but infectious virus to transit to the gut after swallowing, resulting in intestinal infection and fecal shedding.

Respiratory BCoV Pathogenesis in Feedlot Calves

The BRDC of feedlot calves is characterized by fever, dyspnea, inflammatory and necrotizing lung lesions leading to bronchopneumonia, weight loss, and death. It is most pronounced during the first weeks after arrival at feedlots and overlaps with a high prevalence of respiratory viral and secondary bacterial coinfections and various environmental or host stress factors during this period. A growing number of reports in the past decade have provided epidemiologic or experimental evidence suggesting that BCoV respiratory infection contributes substantially to the BRDC. Multiple previous studies (see section on Epidemiology) have documented nasal and fecal shedding of BCoV by calves shortly after arrival in feedlots and subsequent seroconversion to BCoV (see **Table 1**). Storz and colleagues[19] showed a progression in development of the BRDC for natural cases, initiated by BCoV respiratory infection (nasal shedding) upon arrival followed by dual infections with BCoV and respiratory bacteria (*Mannheimia hemolytica* and *Pasteurella multocida*). This progression led to pneumonia and deaths in 26 cases, most of which had concurrent high titers of BCoV and bacteria in the lungs. The cattle died within 5 to 36 days after the disease onset. Clinical signs included high fever and severe respiratory distress. Gross lung lesions consisted of subacute exudative and necrotizing lobar pneumonia involving 50%–80% of the lung volume. Histologic lung lesions were characterized as fibrinous, necrotizing lobar pneumonia, but with moderate to severe bronchitis and bronchiolitis. The investigators concluded that these data supported a role for BCoV in the BRDC as defined by Evans' criteria for causation. In this and another study,[20] researchers confirmed the presence of BCoV antigen in respiratory epithelial cells or isolated BCoV from nasal secretions, trachea, bronchi, or lung alveoli and documented the presence of interstitial emphysema, bronchiolitis and alveolitis in concert with bacterial infection (see **Table 1**). Although BRDC is recognized as a multifactorial disease, this evidence supports a role for BCoV in inciting the disease. Multifactorial/multiagent experiments describing interactions between respiratory viruses, bacteria, and stress have highlighted their synergistic effects in the BRDC[70] but no such experiments have been reported for BCoV. Such studies of feedlot age, BCoV-seronegative stressed calves are needed to further elucidate the role of BCoV in the BRDC and the mechanisms involved in predisposing calves to development of fatal pneumonia.

Cofactors that Exacerbate Respiratory BCoV Infections, Disease, or Shedding

Underlying disease or respiratory coinfections, dose and route of infection, and immunosuppression (corticosteroids) are all potential cofactors that can exacerbate the severity of BCoV infections and enhance virus transmission or host susceptibility. The BRDC (shipping fever) is recognized as a multifactorial, polymicrobial respiratory disease complex in young adult feedlot cattle, with several factors exacerbating respiratory disease, including BCoV infections.[13,17–21,23,25,49] The BRDC can be precipitated by several viruses, alone or in combination (BCoV, bovine respiratory syncytial virus, parainfluenza-3 virus, bovine herpesvirus), and viruses capable of mediating immunosuppression (eg, bovine viral diarrhea virus). The shipping of cattle long distances to feedlots and the comingling of cattle from multiple farms creates physical stresses that overwhelm the animal's defense mechanisms and provide close contact for exposure to high concentrations of new pathogens or strains not previously encountered. For the BRDC, various predisposing factors (viruses, stress) allow commensal bacteria of the nasal cavity (eg, *Mannheimia haemolytica*, *Pasteurella* sp, *Mycoplasma* sp) to infect the lungs leading to a fatal fibrinous pneumonia.[17,19–21]

It is also possible that antibiotic treatment of animals coinfected with BCoVs and bacteria, with massive release of bacterial lipopolysaccharides (LPS), could precipitate induction of proinflammatory cytokines, which may further enhance lung damage. Van Reeth and colleagues[71] showed that pigs infected with porcine respiratory CoV followed by a subclinical dose of LPS from *Escherichia coli* within 24 hours developed enhanced fever and more severe respiratory disease compared with each agent alone. The investigators concluded that the effects were likely mediated by the significantly enhanced levels of proinflammatory cytokines induced by the bacterial LPS in synergy with the CoV infection. Thus, there is a need to examine LPS and lung cytokine levels in BCoV-infected feedlot cattle as possible mediators of the severity of BRDC.

Corticosteroids induce immunosuppression and reduce the numbers of CD4 and CD8 T cells and certain cytokine levels.[72–74] A recrudescence of BCoV fecal shedding was observed in 1 of 4 WD-BCoV-infected cows treated with dexamethasone.[6] Similarly, treatment of weaned pigs with dexamethasone before porcine respiratory CoV inoculation led to increased disease severity and reduced T-cell immune responses in the treated pigs.[73,74]

IMMUNITY AND VACCINES

An understanding of the pathogenesis of respiratory BCoV infections including the target organs infected and how virus is disseminated to these organs should assist in development of vaccine strategies. The realization that BCoV infects epithelial cells lining the respiratory and/or intestinal tracts may necessitate development of vaccines effective at both sites of infection to provide optimal protection. In addition, vaccines for mucosal pathogens may fail to induce sterilizing immunity or to prevent respiratory reinfections, as observed for natural or experimental respiratory BCoV infections.[16,29] Consequently, the major focus for vaccine should be to prevent the severe disease-requiring treatments and the weight loss/reduced gain in the infected animals. These objectives may be best accomplished by vaccinating calves on farm before shipping to auction barns or feedlots because BCoV infections occur at the auction barn or shortly after arrival at feedlots necessitating rapid onset of immunity.

Despite its economic impact, no respiratory BCoV vaccines have been developed to prevent BCoV-associated pneumonia in young calves or in the BRDC of feedlot cattle. The correlates of immunity to respiratory BCoV infections remain undefined. Limited

data from epidemiologic studies of BCoV infections in cattle suggest that serum antibody titers to BCoV may be a marker for respiratory protection. In multiple studies the BCoV antibody isotype (IgG1, IgG2, IgA), neutralizing and HI antibody titer, and magnitude of antibody titer in serum of naturally infected calves or in cattle on arrival in feedlots were correlated with protection against respiratory disease, pneumonia, or BCoV respiratory shedding.[13,24,25,39,50,51,69] However, whether the serum antibodies are themselves correlates of respiratory protection or only reflect previous enteric or respiratory exposure to BCoV is uncertain and requires additional research. If serum BCoV neutralizing antibodies are indeed a correlate of immunity to respiratory BCoV infection,[51] then parenteral vaccines to boost levels of existing BCoV antibodies may be protective.

Lin and colleagues[51,69] assessed the kinetics of the neutralizing and HI antibody responses to respiratory BCoV in sera of cattle during an epizootic of pneumonia associated with the BRDC. Cattle shedding respiratory BCoV at the start of the epizootic had low levels of neutralizing and HI antibodies, whereas cattle with high levels of antibodies against the HE and S viral glycoproteins remained negative for respiratory BCoV. The neutralizing and HI-BCoV antibody levels in serum were highly correlated. Moreover all the BCoV antibody isotypes measured (IgM, IgG1, and IgG2) were significantly correlated with the levels of neutralizing and HI antibodies. In cattle with fatal respiratory BCoV infections, only IgM antibody responses were detected.

Alternatively, in a recent study, IN vaccination of feedlot calves with a modified live-BCoV calf vaccine on entry to a feedlot reduced the risk of treatment for BRDC in calves.[49] The immune correlates for the BCoV vaccine were not identified, but based on data from IN-modified live infectious bovine rhinotracheitis vaccines, the investigators speculated that locally induced interferon or IgA antibodies could be involved. The data further suggest that IN administration of a BCoV CD strain induces cross-protection against field exposure to a respiratory BCoV. The findings concur with results of experimental challenge studies of calves confirming cross-protection among BCoV strains of distinct clinical origin. Inoculation of gnotobiotic or colostrum-deprived calves with CD, WD, or respiratory BCoV strains led to nasal and fecal CoV shedding followed by complete cross-protection against diarrhea after challenge with a calf diarrhea strain.[29,30] Subclinical nasal and fecal virus shedding (detected only by RT-PCR) in calves challenged with the heterologous BCoV strains[29,30] confirmed field studies documenting subclinical BCoV infections and the potential for subclinically infected animals to be a reservoir for BCoV in infected herds.[16,24] Consequently, the goal is to develop vaccines that are protective against divergent field strains of BCoV, including ones from the distinct clinical syndromes. Inclusion of a mixture of strains representing CD, WD, and respiratory isolates may be an ideal strategy to develop a single broad-spectrum BCoV vaccine effective against BCoV infections associated with each of the clinical syndromes including respiratory disease.

FUTURE RESEARCH

Future areas for research include understanding the basic mechanisms of how BCoVs induce a broad spectrum of respiratory disease (ranging from mild to fatal) and the cofactors and interactions that exacerbate disease or lead to enhanced shedding and transmission. Relatively little is known about the basis of interspecies transmission of BCoVs or why they have broad host ranges. Little is understood about the ecology of bovine-like CoVs present in wildlife reservoirs or their potential to emerge as either public or animal health threats. A lack of basic understanding of the attributes

required for effective respiratory BCoV vaccines and the immune correlates of protection remain obstacles to vaccine development.

SUMMARY

- BCoV causes pneumoenteric infections in cattle and wild ruminants. Detection of similar CoV strains among wild ruminants and in dogs, with evidence for experimental interspecies transmission to calves, suggests that these species could harbor CoVs transmissible to cattle or vice versa.
- BCoV is associated with 3 clinically distinct syndromes in cattle: CD, WD in adults, and respiratory disease in cattle of various ages.
- Greater biologic, antigenic, and genetic diversity is evident between contemporary and historic BCoV strains than between enteric and respiratory strains. There are no consistent markers to discriminate between respiratory and enteric BCoVs.
- Existence of a single serotype of BCoV and cross-protection among strains from the different clinical syndromes suggests that a single broadly cross-reactive strain of BCoV may suffice for a vaccine. Alternatively, because of variations among field strains, including ones from the 3 clinical syndromes, a single vaccine composed of CD, WD, and respiratory BCoV isolates may provide a broader spectrum of protection against all 3 syndromes.
- An accumulating body of evidence suggests that BCoV respiratory infections contribute substantially to the BRDC of feedlot cattle.
- Underlying disease or respiratory coinfections, dose and route of infection (fecal/oral or aerosols), and immunosuppression (corticosteroids) related to shipping or immunosuppressive viral coinfections are potential cofactors that could exacerbate the severity of BCoV infections and the BRDC.
- Samples for antemortem diagnosis of BCoV infections include nasal swabs, tracheobronchial aspirates, and feces collected in the acute stage of infection; postmortem tissues include ones from the upper respiratory tract, lung, ileum, and colon. Highly sensitive molecular assays (RT-PCR, nested RT-PCR, qRT-PCR) are being used increasingly to detect BCoV RNA in diagnostic samples including tissue homogenates (lung, and so forth).
- Because most cattle are seropositive to BCoV, in suspect herd outbreaks, collection and testing of paired sera for BCoV antibody tests is essential for diagnosis.
- There are no BCoV vaccines to prevent respiratory BCoV infections in cattle, and the correlates of immunity to respiratory BCoV infections are unknown.
- In multiple studies, the serum BCoV antibody isotype (IgG1, IgG2, IgA), neutralizing and HI antibody titer, and magnitude of antibody titer in serum of cattle on arrival in feedlots were correlated with various parameters of protection against the BRDC. Whether the serum antibodies are correlates of protection or only reflect previous BCoV exposure is uncertain.
- Because BCoV infections commonly occur at or shortly after arrival of cattle to feedlots or at auction barns, use of BCoV vaccines in preconditioning calf programs before shipment of calves would be the most beneficial.

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