Bovine Coronavirus

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35.1 INTRODUCTION

Bovine coronavirus (BCoV) is a single-stranded, positive-sense RNA virus implicated in three distinct clinical syndromes in cattle: (1) calf diarrhea (CD), (2) winter dysentery (WD) with hemorrhagic diarrhea in adults, and (3) bovine respiratory disease complex (BRDC) or shipping fever of feedlot cattle. Given its common occurrence in dairy and beef cattle farms worldwide and its negative impact on milk production in dairy herds and weight gain in calves and adult cattle, considerable efforts have been made in recent years toward the elucidation of BCoV’s genetic characteristics, epidemiology, transmission, clinical features, pathogenesis, diagnosis, treatment, and prevention.

35.1.1 CLASSIFICATION

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses classified in the family Coronaviridae, order Nidovirales. Of the two subfamilies within the family Coronaviridae, the subfamily Coronavirinae is separated into four genera, Alphacoronavirus and Betacoronavirus naturally occurring in bats and Deltacoronavirus and Gammacoronavirus residing primarily in birds and pigs, while the subfamily Torovirinae is divided into two genera, Bafinivirus and Torovirus, being present in fish and mammals (causing gastroenteritis in mammals such as cattle, horses, and pigs, but rarely humans), respectively [1].

Currently, the genus Alphacoronavirus consists of at least 16 species: transmissible gastroenteritis virus, porcine respiratory CoV, canine CoV, feline infectious peritonitis virus, mink CoV, rhinolophus bat CoV HKU2, miniopterus bat CoV 1A, miniopterus bat CoV 1B, miniopterus bat CoV HKU8, human CoV 229E (HCoV-229E), human CoV NL63 (HCoV-NL63), porcine epidemic diarrhea virus (see Chapter 37), scotophilus bat CoV 512, hipposideros bat CoV HKU10, roussettus bat CoV HKU10, and mystacina tuberculata bat CoV [2].


The genus Deltacoronavirus contains wigeon CoV HKU20, common moorhen CoV HKU21, night heron CoV HKU19, bulbul CoV HKU11 (type species), munia CoV HKU13, magpie–robin CoV HKU18, thrush CoV HKU12, white-eye CoV HKU16, porcine CoV HKU15, and sparrow CoV HKU17 [2].

The genus Gammacoronavirus consists of infectious bronchitis virus (IBV), partridge CoV (IBV-partridge), turkey CoV, peafowl CoV (IBV-peafowl), beluga whale CoV SW1, and bottlenose dolphin CoV HKU22 [2].
The genus *Bafinivirus* contains a single species white bream virus and the genus *Torovirus* consists of equine torovirus (type species; formerly Berne virus), human torovirus, porcine torovirus, and bovine torovirus (BoTV; formerly Breda virus, BRV). Of particular relevance, BoTV is a kidney-shaped virus associated with diarrhea in calves worldwide (see Chapter 38) [1].

Interestingly, although coronaviruses in the subfamily *Coronavirinae* do not usually produce clinical symptoms in their natural hosts (bats and birds), accidental transmission of these viruses to humans and other animals may result in respiratory, enteric, hepatic, or neurologic diseases of variable severity. Coronaviruses that have been shown to cause disease in humans include HCoV-229E (alphacoronavirus 1b), HCoV-NL63 (alphacoronavirus 1b), HCoV-OC43 (betacoronavirus 2a), HCoV-HKU1 (betacoronavirus 2a), SARS-CoV (betacoronavirus 2b), and MERS-CoV (betacoronavirus 2c) [2].

Belonging to Lineage A, genus *Betacoronavirus*, subfamily *Coronavirinae*, and family *Coronaviridae*, BCoV consists of a single serotype that encompasses a diversity of strains/isolates (e.g., BCoV Bubalus, BCoV cow, BCoV alpaca, bovine enteric coronavirus, bovine respiratory coronavirus, human enteric coronavirus 4408, sambar deer coronavirus, waterbuck coronavirus, and white-tailed deer coronavirus) that demonstrate genetic and antigenic proximities to HCoV-OC43, PHEV, and ECoV. Besides inducing diarrhea in newborn calves, WD, with hemorrhagic diarrhea in adult cattle, and respiratory tract infections in calves and feedlot cattle, BCoV has the capacity to cause transboundary infection/disease in buffalos, lamas, alpacas, deer, and giraffes [6].

Phylogenetic examination of BoCV isolates reveals the existence of two genomic clades (1 and 2). While reference enteric BoCV strains and a vaccine strain belong to clade 1, BCoV from respiratory tract, nasal swab, and bronchoalveolar washing fluids belongs to clade 2. In addition, the respiratory isolates from Oklahoma are further separated into three subclades, 2a, 2b, and 2c [7,8].

### 35.1.2 Morphology and Genome Organization

BCoV possesses an enveloped, pleomorphic/spherical virion of 65–210 nm in diameter, with a double layer of short (hemagglutinin) and long (spike) surface projections, the latter of which appear club-shaped and measure about 20 nm in length. The lipoprotein envelope carries four structural proteins: membrane (M) glycoprotein, spike (S) glycoprotein, hemagglutinin–esterase (HE) glycoprotein, and small envelope (E) protein. Beneath the envelope is a flexible and helical nucleocapsid formed by nucleoprotein (N) in association with the viral genome.

The genome of BCoV is a linear, nonsegmented, single-stranded, positive-sense RNA of 31,028 nt and includes 13 open reading frames (ORFs) flanked by 5′- (nt 1–210) and 3′- (nt 30,740–31,028) untranslated regions (UTRs). Its 5′-UTR is capped, and its 3′-UTR contains a polyadenylated tail. Some over-lappings are observed in ORF1a (nt 211–13,362, including stop codon) and ORF1b (13,332–21,494); ORF4 (S, 23,641–27,732) and ORF5 (N, “4.9 kDa”, 27,722–27,811); ORF7 (N, “12.7 kDa”, 28,106–28,435) and ORF8 (E, 28,422–28,676); and ORF10 (N, 29,393–30,739) and ORF11 (I, 29,454–30,077). Intergenic sequences are identified in ORF1b and ORF2 (N, 32 kDa, 21,504–22,340); ORF2 and ORF3 (HE, 22,352–23,626); ORF3 and ORF4; ORF5 and ORF6 (N, “4.8 kDa”, 27,889–28,026); ORF6 and ORF7; ORF8 and ORF9 (M, 28,691–29,383); and ORF9 and ORF10 [9].

Comparison of genome sequences of two field isolates of BCoV, representing respiratory (BCoV-R) and enteric (BCoV-E) strains, respectively, revealed differences in 107 out of 31,028 positions, scattered throughout the genome except the 5′-UTR. Differences in 25 positions are nonsynonymous, leading to 24 amino acid changes in all proteins except p11b. The remaining 82 nucleotide differences do not cause amino acid changes, although they might modulate phenotypic properties by affecting the RNA structure and/or RNA interaction(s). Six replicate mutations are identified within or immediately downstream of the predicted largest pp1a-derived protein, p195/p210. It is possible that single amino acid changes within p195/p210 as well as within the S glycoprotein may have contributed to the different phenotypes of the BCoV isolates [9].

Of the five structural proteins (nucleocapsid [N], transmembrane, small envelope [E], hemagglutinin–esterase [HE], and spike [S] proteins) encoded by the BCoV genome, the N protein (50 kDa) is highly conserved and offers an ideal target for molecular diagnostic application; the surface HE glycoprotein (120–140 kDa) acts a receptor destroying enzyme (esterase) to reverse hemagglutination; and the outer surface S glycoprotein (190 kDa, 1363 aa) forms large club (petal)-shaped spikes on the surface of the virion. Cleavage of the S protein by an intracellular protease between aa 768 and 769 results in a variable S1 N-terminal domain (subunit) and a more conserved S2 C-terminal domain (subunit). The S1 subunit is involved in virus binding to host–cell receptors, induction of neutralizing antibody expression, and hemagglutinin activity. With a highly variable/mutable sequence, the S1 is associated with changes in antigenicity, tissue tropism, viral pathogenicity, and host range [10]. Indeed, a single amino acid change (A528V) within the BCoV S1 subunit has been shown to confer resistance to VN [11]. The S2 is the transmembrane subunit with a critical role in the mediation of fusion between viral and cellular membranes. Interestingly, no consistent differences are observed in the full-length S gene between BCoV strains causing respiratory and enteric diseases [12].

### 35.1.3 Biology and Epidemiology

BCoV has a predilection for intestinal and pulmonary epithelial cells. First recognized as enteric pathogens (BCoV-E) in association with CD and WD in adult cattle, BCoV was also identified as respiratory pathogens (BCoV-R) in association with BRDC or shipping fever of feedlot cattle. Although BCoV strains causing enteric (BCoV-E) or respiratory (BCoV-R) infection may show distinct biological properties and host cell tropism, they are closely related antigenically.
and genetically [13–17]. In fact, some BCoV strain may cause simultaneous enteric and respiratory tract infections.

Transmission of BCoV is mainly through fecal–oral and respiratory (aerosol) routes. As in the case of BRDC, BCoV may enter the host via aerosol and undergo initial and extensive replication in the nasal mucosa. Following ingestion of mucus secretions containing large quantities of infectious virus with protective coating, cattle with respiratory infection may develop gastrointestinal symptoms such as diarrhea. Clinically or subclinically infected calves and young adult cattle function as reservoirs for BCoV in the herd, with sporadic shedding of the virus in feces and nasal secretions [18].

BCoV has a widespread presence in both dairy and beef cattle. WD has been described in Europe, North America, and East Asia, with a peak incidence in winter, while CD and shipping fever are found in various regions of the world [19–21]. Although BCoV outbreaks typically occur in autumn and winter, severe cases of infection in adult cattle may also emerge in warmer seasons. Economic losses due to BCoV infection are attributable to dramatic reduction in milk yield in dairy herds and loss of body condition in both calves and adults, with death being a not uncommon outcome for affected calves.

Unlike many other coronaviruses that have restricted host ranges, BCoV has the ability to cause transboundary infections. This is evidenced by the findings that bovine-like CoV is present in sambar deer (Cervus unicolor), white-tailed deer (Odocoileus virginianus), waterbuck (Kobus ellipsiprymnus), elk (Cervus elephas), caribou (Rangifer tarandus), giraffe, water buffalo calves, alpacas, and dogs [22–27], and that bovine-like CoV is implicated in deer and waterbuck with bloody diarrhea resembling WD in cattle. Furthermore, isolation of a BCoV-like human enteric coronavirus-4408/US/94 from a child with acute diarrhea highlights the public health impact of BCoV.

As an enveloped virus, BCoV is sensitive to detergents and lipid solvents such as ether and chloroform. It is also inactivated by conventional disinfectants, formalin, and heat [28].

35.1.4 Clinical Features and Pathogenesis

With an incubation of 3–8 days, BCoV is implicated in enteric disease in newly born calves (CD) and adult cattle (WD) as well as respiratory tract infections of growing calves and shipping fever pneumonia in feedlot cattle. Although BCoV infection rarely causes death, its morbidity can be as high as 100%, contributing to sudden and dramatic reduction in milk production and loss of body weight.

35.1.4.1 CD (calf scours)

BCoV infection in calves between the ages of 1 and 3 weeks often produces gastrointestinal signs such as profuse diarrhea (watery yellow, gray, or greenish containing blood or mucus), dehydration, depression, weakness, weight loss, anorexia, convulsions, and sometimes death. Notable lesions include colonic villous atrophy. Some calves (2–6 months of age) may develop respiratory infection with a serous to purulent nasal discharge, coughing, rhinitis, pneumonia, fever, and inappetence, often with concurrent diarrhea. Sporadic shedding of virus in nasal secretions and feces may begin 5 days after infection. Secondary bacterial infections with other common respiratory viruses (e.g., bovine rotavirus A), bacteria, and mycoplasms may exacerbate clinical signs [2,29].

WD with hemorrhagic diarrhea in adults: BCoV infection in adults is normally subclinical, although it may cause WD outbreaks in housed cattle over the winter months, with a sudden onset of semiliquid, often hemorrhagic diarrhea, leading to severe anemia, marked drop in milk production in dairy herds, and death. Histopathologically, loss of surface epithelial cells and necrosis of crypt epithelial cells in the large intestine containing detectable virus particles are noted [30–32].

BRDC or shipping fever: BCoV infection in young adult feedlot cattle (6–10 months of age) may develop fever, coughing, dyspnea, rhinitis, bronchopneumonia, anorexia, diarrhea, and weight loss, accompanied by necrotizing lung lesions (e.g., interstitial emphysema, bronchiolitis, and alveolitis). Death of infected cattle may occur as a result of necrotizing pneumonia. Shedding of respiratory BCoV in nasal secretions and feces begins 4–10 days after infection, with nasal shedding consistently preceding fecal shedding. Interestingly, cattle with relatively high respiratory BCoV antibody ELISA titers or neutralizing antibodies in serum are less likely to shed respiratory BCoV [33,34].

The pathogenesis of BCoV-induced diarrhea lies in its ability to destroy surface epithelial cells of the intestinal villi in both the small and large intestines, with remarkable loss of absorptive capacity, increase in the gut fluid volume, and osmotic imbalance. This contributes to rapid loss of water and electrolytes, hypoglycemia, lactic acidosis, hypervolemia, acute shock, cardiac failure, and death. The severity of the disease is influenced by the host age, nutritional and immune status, virus load, stresses, and copresence of other pathogenic organisms. For example, the onset of BRDC may be linked to coinfection with bovine respiratory syncytial virus, parainfluenza-3 virus, bovine herpesvirus, and bovine viral diarrhea virus that have the capacity to sabotage host’s immune mechanisms. In addition, long-distance shipping with cattle from multiple feedlots may create physical stresses that compromise host’s immune defense against BCoV and other pathogens, and also increase exposure of cattle to high concentrations of new pathogens not previously encountered. Chemotherapy (e.g., corticosteroids and dexamethasone) may reduce the numbers of cells (CD4 and CD8 T cells) and levels of cytokines that directly impact on host immune fitness.

35.1.5 Diagnosis

BCoV is associated with CD, WD in adults, and BRDC (shipping fever). Diagnosis of BCoV infection requires differentiation from other diseases with similar symptoms. For example, bovine rotavirus A (BRV), Escherichia coli, Salmonella enterica, Giardia intestinalis, and Cryptosporidium parvum are known to cause diarrhea in calves, while Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,
Arcanobacterium pyogenes, and Mycoplasma are often involved in bovine respiratory disease [35,36].

Although a presumptive diagnosis can be made on the basis of history and clinical signs, definitive diagnosis of BCoV infection relies on laboratory confirmation of the presence of virus particles/antigens and antiviral antibodies in nasal secretions, feces, and tissues.

Virus isolation using the G clone of human rectal tumor (HRT)-18 cells, or Vero cells, provides a useful way to diagnose BCoV infection [37].

Electron microscopy allows direct observation of BCoV particles in feces and other samples. Typically, 5 g of feces is resuspended in 25 mL of phosphate buffered saline (PBS) and centrifuged at 5000 × g for 20 min. The clarified supernatant (12 mL) is centrifuged again at 100,000 × g for 1 h, and the resultant pellet is resuspended in 1 mL of water. Then, 5 µL of sample is mixed with 5 µL of 2% phosphotungstic acid (pH 6.9), and 1 µL of the mixture is applied to the center of a 300-mesh grid. The sample is side blotted with a piece of torn filter paper to remove the majority of the sample, thus leaving a fine layer to air dry. Samples are examined under a microscope at 30,000× magnification [10].

Serological assays such as direct fluorescent antibody test, virus neutralization (VN), hemagglutination inhibition, and enzyme-linked immunosorbent assay (ELISA) enable detection of viral antigens and antiviral antibodies in nasal secretions, feces, serum, and frozen or paraffin-embedded tissues (trachea, lung, ileum, and colon) [10].

Nucleic acid amplification techniques such as PCR allow sensitive detection of viral nucleic acids directly from clinical specimens [38–46]. In particular, real-time reverse transcription (RT)-PCR targeting conserved regions of the BCoV genome (polymerase or N protein) facilitates rapid detection of divergent strains [47,48]. In addition, sequence analysis of the S gene (polymerase or N protein) facilitates rapid detection of divergent strains [47,48].

35.1.6 TREATMENT AND PREVENTION

Treatments of animals with BCoV infections are largely aimed at alleviation of clinical symptoms, including intravenous fluid therapy and provision of adequate colostomy intake in newborn calves. In addition, isolation of calves with diarrhea and ventilation of housing are helpful in reducing disease incidence.

Control of BCoV-related diarrhea is possible by vaccinating calves on farm prior to shipping to auction barns or feedlots with a live vaccine (ATCvet code Q102). Although no vaccines are currently available to prevent BCoV-associated pneumonia in young calves or in the BRDC of feedlot cattle, there is evidence that intranasal (IN) vaccination of feedlot calves with a modified live BCoV calf vaccine on entry to a feedlot reduces the risk of treatment for BRDC in calves. In addition, IN administration of a BCoV CD strain induces cross-protection against field exposure to a respiratory BCoV [54,55]. Future research on the combined use of strains representing CD, WD, and respiratory isolates may open a way for a single broad-spectrum BCoV vaccine against BCoV infections [10].

35.2 METHODS

35.2.1 SAMPLE PREPARATION

Samples (feces, nasal swabs, and nasal swabs) are collected from suspected cases of gastrointestinal and/or respiratory tract infections. Swabs are placed immediately after collection in 2 mL minimum essential media (MEM) containing 1000 U penicillin and 1 mg streptomycin. After transport on dry ice, samples are mixed by pulse-vortexing for 15 s, and swabs are discarded. Sample suspensions are diluted 1:10 in MEM and centrifuged at 5000 rpm for 15 min at 4°C. The clarified supernatants are transferred to sterile vials, and aliquots of 140 µL are separated for RNA extraction using QIAamp Viral RNA Extraction Kit (Qiagen).

Fecal suspensions (approximately 0.1 g of feces or 100 µL in 1 mL 1× PBS, pH 7.5–8.0) are mixed for 1 min at 15 Hz on a tissue lyser. Fecal suspension of 50 µL is mixed with 400 µL of lysis solution, 1 µL of carrier RNA (1 µg/µL), and 1 µL of exogenous internal positive control (XIPC) RNA (at 10,000 copies/µL) for 5 min at 15 Hz and centrifuged at 20,000 × g for 3 min to generate the clarified lysate. The clarified lysate (350 µL) is transferred to a 96-well, deep-well plate containing 20 µL of magnetic bead mix (10 µL of lysis/binding enhancer and 10 µL of RNA binding beads), and 200 µL of isopropanol is added. The plate is loaded onto an automated magnetic particle processor for nucleic acid purification: lysis/binding for 5 min, one 2 min wash 1, one 2 min wash 2, 1 min dry step, and a 3 min heated elution step at 70°C. Purified nucleic acid is then denatured at 95°C for 5 min in a thermal cycler to denature the double-stranded BRV RNA for RT-PCR.

Alternatively, feces are diluted in nuclelease-free water (Promega) at a ratio of 3:1 (v/v), and suspensions are centrifuged at 5000 × g for 10 min at 4°C. RNA is extracted from 250 µL of supernatant recovered using the TRIzol Reagent (Invitrogen), and the RNA pellet is diluted in 30 µL of nuclelease-free water (Promega).

Tissues from the upper respiratory tract (nasal, pharyngeal tissues, trachea) and lung, and tissues from the distal small intestine and colon may be collected from suspect necropsy disease cases. RNA is extracted from these tissues by using Qiagen RNeasy Kit.

35.2.2 DETECTION PROCEDURES

35.2.2.1 Conventional RT-PCR

Erlas et al. [56] reported a gel-based RT-PCR for the detection of BCoV RNA in clinical samples with primers from the spike-protein gene (Sp1: 5′-CTTATAAGT GCCCCCAAACTAAA-3′, nt 25,277–25,300 and Sp2: 5′-CC TACTGTGAGATCACATGTTTG-3′, nt 25,876–25,898), which generate a specific product of 622 bp.

RT-PCR is conducted with SuperScript™ One-Step RT-PCR Kit (Invitrogen) using the following cycling program: RT at 50°C for 30 min, inactivation of Superscript II RT at 94°C for 2 min, and 45 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 30 s, with a final extension at 68°C.
for 10 min. PCR products are separated by electrophoresis in 1.5% agarose gels and visualized under UV light after ethidium bromide staining [56,57].

35.2.2.2 Real-Time RT-PCR
Cho et al. [58] utilized primers BCVF and BCVR from the spike (S) gene sequence for specific detection of BCoV. The TaqMan probe BCVPb is labeled with 6-carboxyfluorescein (FAM) at the 5′ end and with a nonfluorescent quencher 1 (NFQ1) at the 3′ end (Table 35.1).

RT-PCR mixture (25 μL) is prepared with AgPath-ID™ Multiplex RT-PCR Kit (Applied Biosystems), containing 5 μL extracted template, 500 nM each of BCVF and BCVR, and 150 nM BCVPb.

Amplification and detection are carried out on an automated real-time PCR system (Smart Cycler® II, Cepheid, Inc.) with the following cycling parameters: 50°C for 30 min, 95°C for 15 min, and 35 cycles of 94°C for 15 s and 60°C for 60 s.

Samples with a Ct of 35 cycles or less are considered positive.

### REFERENCES


