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MINIREVIEW

Comparative Pathogenesis of Bovine and Porcine Respiratory Coronaviruses in the Animal Host Species and SARS-CoV-2 in Humans

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ABSTRACT Discovery of bats with severe acute respiratory syndrome (SARS)-related coronaviruses (CoVs) raised the specter of potential future outbreaks of zoonotic SARS-CoV-like disease in humans, which largely went unheeded. Nevertheless, the novel SARS-CoV-2 of bat ancestral origin emerged to infect humans in Wuhan, China, in late 2019 and then became a global pandemic. Less than 5 months after its emergence, millions of people worldwide have been infected asymptomatically or symptomatically and at least 360,000 have died. Coronavirus disease 2019 (COVID-19) in severely affected patients includes atypical pneumonia characterized by a dry cough, persistent fever, and progressive dyspnea and hypoxia, sometimes accompanied by diarrhea and often followed by multiple organ failure, especially of the respiratory and cardiovascular systems. In this minireview, we focus on two endemic respiratory CoV infections of livestock: bovine coronavirus (BCoV) and porcine respiratory coronavirus (PRCV). Both animal respiratory CoVs share some common features with SARS-CoV and SARS-CoV-2. BCoV has a broad host range including wild ruminants and a zoonotic potential. BCoV also has a dual tropism for the respiratory and gastrointestinal tracts. These aspects, their interspecies transmission, and certain factors that impact disease severity in cattle parallel related facets of SARS-CoV or SARS-CoV-2 in humans. PRCV has a tissue tropism for the upper and lower respiratory tracts and a cellular tropism for type 1 and 2 pneumocytes in lung but is generally a mild infection unless complicated by other exacerbating factors, such as bacterial or viral coinfections and immunosuppression (corticosteroids).

KEYWORDS animal coronaviruses, bovine respiratory coronavirus, COVID-19, pathogenesis, porcine respiratory coronavirus, SARS, SARS-CoV-2

Coronaviruses (CoVs) are enveloped, pleomorphic, and 60 to 220 nm in diameter, including the club-shaped spike (S) glycoproteins that are approximately 12 to 25 nm in length. CoVs contain a single-stranded positive-sense RNA genome of 26 to 32 kb (1). CoVs exist as quasispecies and have high rates of mutation and recombination (2, 3). This fosters the emergence of new CoV strains with altered cell tropisms and host specificity and propels their interspecies transmission to infect new animal and human hosts. Severe acute respiratory syndrome coronavirus (SARS-CoV), the predecessor of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), originated from a bat ancestor and spread via an intermediate animal host (civet cats) to cause zoonotic disease in humans (4, 5). The epidemic began in China in late 2002, and within 6 months, it had spread rapidly to more than 30 countries. The global spread was contained in July 2003 after more than 8,422 cases and 916 deaths, with a case fatality rate of 11% (6). The discovery of bats with SARS-related CoVs (4, 7) raised the specter of potential future outbreaks of zoonotic SARS-CoV-like disease in humans, which largely went unheeded. Nevertheless, the novel SARS-CoV-2 of bat ancestral origin

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TABLE 1 Coronaviruses (CoVs) in domestic livestock or poultry and associated diseases

Host	CoV genus	CoV subgenus	CoV common name	Major associated disease
Pig	AlphaCoV	*Tegacovirus*	Transmissible gastroenteritis virus (TGEV)	Gastroenteritis
	AlphaCoV	*Tegacovirus*	Porcine respiratory coronavirus (PRCV)	Respiratory disease
	AlphaCoV	*Pedacovirus*	Porcine epidemic diarrhea virus (PEDV)	Gastroenteritis
	AlphaCoV	*Rhinacovirus*	Severe acute diarrhea syndrome coronavirus (SADS-CoV)	Gastroenteritis
	BetaCoV	*Embecovirus*	Porcine hemagglutinating encephalomyelitis virus (pHEV)	Neurological and/or enteric disease
	DeltaCoV	*Buldecovirus*	Porcine deltacoronavirus (PDCoV)	Gastroenteritis
Ruminants				
Alpaca	AlphaCoV	*Duvinacovirus*	Alpaca alphacoronavirus (ACoV)	Respiratory disease
Dromedary camel	AlphaCoV	*Duvinacovirus*	Dromedary camel alphacoronavirus	Respiratory disease?
Cattle	BetaCoV	*Embecovirus*	Bovine coronavirus (BCoV)	Gastroenteritis and/or respiratory disease
Sheep	BetaCoV	*Embecovirus*	BCoV-like CoV	Gastroenteritis
Goat	BetaCoV	*Embecovirus*	BCoV-like CoV	Gastroenteritis
Llama	BetaCoV	*Embecovirus*	BCoV-like CoV	Gastroenteritis
Alpaca	BetaCoV	*Embecovirus*	BCoV-like CoV	Gastroenteritis and/or respiratory disease
Dromedary camel	BetaCoV	*Embecovirus*	BCoV-like dromedary camel CoV UAE-HKU-23	Gastroenteritis
Dromedary camel	BetaCoV	*Merbecovirus*	Dromedary camel Middle East respiratory syndrome coronavirus (MERS-CoV)	Respiratory disease
Poultry				
Chicken	GammaCoV	*Igacovirus*	Infectious bronchitis virus (IBV)	Respiratory disease, often with multiorgan tissue damage involving kidney, oviduct, and intestinal tract
Turkey	GammaCoV	*Igacovirus*	Turkey coronavirus (TCoV)	Enteric disease
Quail	GammaCoV	*Igacovirus*	Quail coronavirus (QCoV)	Enteric disease
Guineafowl	GammaCoV	*Igacovirus*	Guineafowl coronavirus (GCoV)	Enteric disease

emerged to infect humans in Wuhan, China, in late 2019 and then became a global pandemic (8, 9). Less than 5 months after its emergence, millions of people worldwide have been infected asymptomatically or symptomatically and at least 360,000 have died. Coronavirus disease 2019 (COVID-19) in severely affected patients includes atypical pneumonia characterized by a dry cough, persistent fever, and progressive dyspnea and hypoxia, sometimes with diarrhea and often followed by multiple organ failure (8, 9). Although the virus is more transmissible than SARS-CoV, the overall fatality rate from SARS-CoV-2 infections is less than that for SARS-CoV. However, like SARS and Middle East respiratory syndrome coronavirus (MERS-CoV) (5), COVID-19 is most severe in the elderly and those with comorbidities including chronic health conditions (10). In this minireview, we focus on two endemic respiratory CoV infections of livestock: bovine coronavirus (BCoV) and porcine respiratory coronavirus (PRCV). We review their pathogenesis and factors that impact disease severity in the animal host species and the interspecies transmission and wildlife reservoirs for BCoV in comparison with SARS-CoV or SARS-CoV-2 in humans.

CoV GENERA AND SUBGENERA IN PIGS AND CATTLE

The family *Coronaviridae* in the order *Nidovirales* is composed of four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (1, 11). A total of six swine CoVs have been identified. These include four alphaCoVs, transmissible gastroenteritis virus (TGEV) and PRCV (subgenus *Tegacovirus*), porcine epidemic diarrhea virus (PEDV) (subgenus *Pedacovirus*), and bat HKU2-like swine acute diarrhea syndrome coronavirus (SADS-CoV) (subgenus *Rhinacovirus*); one betaCoV, porcine hemagglutinating encephalomyelitis virus (pHEV) (subgenus *Embecovirus*); and one deltaCoV, porcine deltacoronavirus (PDCoV) (subgenus *Buldecovirus*) (11) (Table 1). In pigs, CoVs affect a variety of organs, including the gastrointestinal (TGEV, PEDV, PDCoV, and SADS-CoV) and respiratory (PRCV) tracts and the peripheral and central nervous systems (pHEV). Together with bovine, human OC43, and canine respiratory CoVs (all

subgenus *Embecovirus*) and SARS-CoV, SARS-CoV-2, and MERS-CoV, pHEV belongs to the genus *Betacoronavirus*. Recently, the two SARS-related CoVs, SARS-CoV and SARS-CoV-2, and MERS-CoV were recognized as subgenera *Sarbecovirus* and *Merbecovirus*, respectively (12). Additional animal CoVs in livestock (swine and ruminant species) and poultry are summarized in Table 1 (1, 11). The detailed etiology or clinical or pathogenic features of the swine or other animal CoVs were also reviewed previously (1, 11, 13–15).

BCoV CAUSES THREE DISTINCT CLINICAL SYNDROMES

BCoV belongs to the *Betacoronavirus* genus (subgenus *Embecovirus* lineage A) of the family *Coronaviridae* (16, 17). BCoV contains a surface S glycoprotein (190 kDa), and like SARS-CoV-2, the S contains a furin cleavage site (18) and is cleaved into 90- and 100-kDa subunits (S1 and S2). Unique to several lineage A betaCoVs, it contains a hemagglutinin esterase (HE), which is a disulfide-linked dimer of 120 to 140 kDa and resembles the hemagglutinin of influenza C virus, that presumably was acquired in a recombination event. Both the S and HE proteins are involved in viral attachment to host cells and induce the formation of neutralizing antibodies to BCoV (16, 17).

BCoV is a pneumoenteric virus that infects the upper and lower respiratory tracts and the intestine and is shed in both feces and upper respiratory tract secretions. This dual tissue tropism mirrors SARS and SARS-CoV-2 infection of not only the respiratory tract but also reportedly the intestine with diarrhea and shedding in stools in some patients (19, 20). BCoV is endemic in cattle worldwide based on antibody seroprevalence data (16, 17, 21, 22). Intriguingly but for undefined reasons associated with the animal age, BCoV causes 3 distinct clinical syndromes in cattle (16, 17, 21, 22): calf diarrhea winter dysentery (WD) with hemorrhagic diarrhea in adults and respiratory infections in cattle of various ages including the bovine respiratory disease complex (BRDC) or shipping fever of feedlot cattle (16, 17, 22, 23). In spite of their association with distinct disease syndromes, all BCoV isolates tested to date from both enteric and respiratory infections are antigenically similar, comprising a single serotype but with 2 to 3 subtypes (16, 17, 21, 22). Although genetic differences (point mutations, but not deletions like PRCV) have been detected in the S gene between enteric and respiratory isolates, including ones from the same animal (24, 25), *in vivo* studies revealed a high level of cross-protection of calves between such isolates (16, 17, 21, 22, 26). Like other CoVs, BCoV represents a quasispecies or swarm of viruses (3, 27), with some viruses potentially more adapted for replication in respiratory versus intestinal sites, possibly contributing to the sequence differences reported for paired enteric/respiratory isolates from the same host (27). Curiously, based on full-length genomic sequences, Zhang et al. (27) 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. Notably, 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) prior to sequencing.

Calf diarrhea. BCoV causes diarrhea in calves 1 to 3 weeks of age when maternal antibodies in milk decline (16, 17, 22, 28, 29). After an incubation period of 3 to 4 days, calves develop a severe, malabsorptive diarrhea persisting for 2 to 8 days. The occurrence of severe diarrhea, resulting in dehydration and death, depends on the BCoV dose, calf age, and calf immune status (16, 17, 22). BCoV infects the epithelial cells of the distal small and large intestine and colon, leading to villous atrophy and crypt hyperplasia. The ensuing malabsorptive diarrhea results in progressive dehydration, acidosis, hyperkalemia, and hypoglycemia that can progress to circulatory failure and death. Concurrent fecal and nasal shedding often occur, and most diarrheic calves necropsied have BCoV antigen in both intestinal and upper respiratory (turbinates, nasal, trachea) epithelial cells (Table 2). Thus, based on experimental challenge studies, enteric strains of BCoV induce diarrhea and are potentially pneumoenteric, but respiratory disease is variable (20% to 30% of calves) (16, 17, 22). Disease is more prevalent in winter, probably due to greater viral stability in the cold, and outbreaks often occur

TABLE 2 Comparison of the respiratory disease and pathology caused by bovine coronavirus (BCoV), porcine respiratory coronavirus (PRCV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and SARS-CoV

Virus	BCoV	PRCV	SARS-CoV-2	SARS-CoV
Genetic group	BetaCoV (subgenus *Embecovirus*)	AlphaCoV (subgenus *Tegacovirus*)	BetaCoV (subgenus *Sarbecovirus*)	BetaCoV (subgenus *Sarbecovirus*)
Host	Cattle	Pig	Human	Human
Origin or possible ancestor	Rat (*China Rattus* CoV HKU24)?	A naturally occurring respiratory variant of transmissible gastroenteritis virus	Bat	Bat
Clinical features in severe symptomatic cases	Fever, coughing, and dyspnea, often with concurrent diarrhea and reduced weight gain as complicated by other factors, such as bacterial or viral coinfections or immune suppression	Fever, atypical pneumonia, and reduced weight gain as complicated by other factors, such as bacterial or viral coinfections or immunosuppression	Fever, atypical pneumonia, acute respiratory distress syndrome (ARDS), and multiple organ failure	Fever, atypical pneumonia, ARDS, and multiple organ failure
Major tissue tropism	Intestinal and/or upper and lower respiratory tract	Upper and lower respiratory tract	Upper and lower respiratory tract	Upper and lower respiratory tract
Cellular tropism in lung	Epithelial cells lining alveoli, possibly type 1 and/or 2 pneumocytes	Type 1 and 2 pneumocytes	Type 1 and 2 pneumocytes	Type 1 and 2 pneumocytes
Acute lung lesions	Bronchoalveolitis; focal emphysema	Bronchoalveolitis	Diffuse alveolar damage (DAD) with exudates	DAD with exudates
Chronic lung lesions	Bronchopneumonia and/or nonsuppurative tracheitis and bronchitis; pulmonary fibrosis	Interstitial pneumonia by type 2 pneumocyte hyperplasia and hypertrophy and infiltration of macrophages and lymphocytes, accompanied by increased inflammatory responses within the lesions	Interstitial pneumonia by type 2 pneumocyte hyperplasia and hypertrophy and infiltration of neutrophils, macrophages, and/or lymphocytes, accompanied by increased inflammatory responses within the lesions and in the blood, and pulmonary fibrosis	Interstitial pneumonia by type 2 pneumocyte hyperplasia and hypertrophy and infiltration of neutrophils, macrophages, and/or lymphocytes, accompanied by increased inflammatory responses within the lesions and in the blood, and pulmonary fibrosis

yearly on the same farm. Within a herd, reservoirs of infection may be clinically infected calves, or subclinically infected calves or cows.

Winter dysentery. Winter dysentery (WD) in adult dairy and beef cattle and in captive wild ruminants is an acute disease characterized by hemorrhagic diarrhea, frequent respiratory signs, anorexia, and decreased milk production in dairy cows (16, 17, 21, 22, 30, 31). Intestinal lesions and BCoV-infected cells in the colonic crypts of cattle resemble those in calf diarrhea, but with extensive necrosis of large intestinal crypt cells and intestinal hemorrhage. In affected herds, morbidity and mortality rates were 20% to 100% and 1% to 2%, respectively. BCoV isolates from WD outbreaks reproduced the disease (bloody diarrhea, decreased milk production) in BCoV-seronegative lactating dairy cows with an incubation period of 3 to 8 days and diarrhea/fecal shedding of 1 to 6 days (32). The cattle developed transient fevers, mild cough, and mucopurulent nasal discharge, consistent with field reports of variable signs of respiratory disease. Of relevance to SARS-CoV-2 antibody seropositives and immunity, BCoV-seropositive nonlactating cattle had transient diarrhea with virus shedding in feces, but without respiratory disease and with nasal shedding in only 20% of cases, suggesting an impact of serum antibodies on the respiratory disease (31). Interestingly, older cattle were more severely affected than similarly exposed calves (32), mimicking the more severe SARS and SARS-CoV-2 cases seen in adults versus children (5, 6). In a subsequent study, calves that originated from a herd with WD showed mild respiratory disease and nasal and fecal BCoV shedding and transmitted BCoV by contact to comingled BCoV-seronegative calves (33). Factors related to the disease manifestations—severe bloody diarrhea, predilection for adult cattle, and winter prevalence—remain enigmas.

Calf respiratory disease. BCoV is implicated as a cause of respiratory disease in both young calves (2 to 6 months) and young adult feedlot cattle (6 to 10 months) (16, 17, 22). In young calves, BCoV is associated with mild respiratory disease (coughing, rhinitis) or pneumonia. Clinical signs include coughing, fever, rhinitis, and dyspnea, often with concurrent diarrhea (Table 2). BCoV has been isolated from nasal and pharyngeal swabs and lung wash of infected calves. Notably, ocular shedding of BCoV in lower titers was also detected in one study (34). This observation aligns with similar findings of eye infections in patients with COVID-19 (35). Experimental calf challenge studies using calf respiratory BCoV isolates confirmed both fecal and nasal shedding and diarrhea, but only variable mild respiratory disease (16, 17, 22, 26). However, in the field, BCoV respiratory infections are likely exacerbated by stress or respiratory coinfections including the common bovine respiratory viruses, bacteria, and mycoplasma species. Of further relevance to the potential for repeated infections or virus shedding in SARS-CoV-2 infections of humans are the long-term longitudinal studies of nasal and fecal shedding of BCoV documenting recurrent nasal shedding. Longitudinal studies of dairy calves from birth to 20 weeks of age documented both fecal and nasal shedding of BCoV, but with diarrhea prominent only in the initial infection (28, 29). Subsequently, recurrent 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 important findings suggest a lack of long-term mucosal immunity in the upper respiratory tract after natural respiratory BCoV infection, confirming similar observations for MERS-CoV infections in seropositive dromedary camels (36) and for human common cold CoVs (37). Also in relation to recurrent viral RNA shedding in some SARS-CoV-2 patients (38), subclinical nasal and fecal viral RNA shedding (detected by RT-PCR, but not ELISA) occurred in calves inoculated with BCoV strains (enteric, respiratory, and WD) and challenged with the heterologous BCoV strain (16, 17, 21, 22, 26), confirming field studies suggesting that subclinically infected animals may be a reservoir for shedding of BCoV in infected herds (28, 29). Interestingly in BCoV-seronegative calves contact-exposed to field calves shedding BCoV from a WD herd, all exposed calves developed mild respiratory disease (with or without diarrhea) (33). The exposed calves shed viral RNA intermittently in feces through day 35, and similarly in nasal secretions through day 28. Viral RNA was detected in medial retro-

pharyngeal lymph nodes and mesenteric lymph nodes through day 42. However, no virus was isolated from nasal swabs after 13 days. Additionally, although viral RNA shedding was present at 21 days when sentinel calves were introduced, they were not infected. The latter is highly relevant to SARS-CoV-2 infections because it shows that prolonged shedding of viral RNA may not reflect virus transmission potential.

Respiratory BCoV associated with the BRDC. The bovine respiratory disease complex (BRDC) is multifactorial disease of 6- to 10-month-old feedlot cattle (shipping fever) consisting of interactions among viral, bacterial, and environmental or host stress factors culminating in respiratory disease. It is characterized by fever, dyspnea, and inflammatory and necrotizing lung lesions leading to bronchopneumonia, weight loss, and death. Respiratory BCoV infections are now recognized as playing an inciting role in the BRDC (16, 17, 22, 23). Multiple studies have documented both nasal and fecal shedding of BCoV shortly after arrival in feedlots following the shipping of cattle from the farm or auction barn. A high percentage of feedlot cattle seroconverted (or had 2- to 4-fold-increased titers) to respiratory BCoV by 3 weeks postarrival. An important observation 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 (16, 17, 22, 39). This suggests that high serum antibody titers coincided with at least some level of protection against BCoV respiratory infection. This is relevant to the question of whether serum antibodies to SARS-CoV-2 are indicative of protective immunity. The development of the BRDC in natural cases is initiated by BCoV infection (nasal shedding) upon arrival followed by dual infections with BCoV and respiratory bacteria (*Mannheimia haemolytica* and *Pasteurella multocida*). This led to high fevers, severe respiratory distress, pneumonia, and deaths in 26 cases (11%), most of which had concurrent high titers of BCoV and bacteria in the lungs (23). BCoV antigen was detected in respiratory epithelial cells, and BCoV was isolated from nasal secretions, trachea, bronchi, or lung alveoli. Lesions included interstitial emphysema, bronchiolitis and alveolitis, necrotic respiratory epithelium, and nonsuppurative inflammatory cell infiltration into the mucosa in concert with the bacterial infection (Table 2).

Respiratory cofactors that exacerbate respiratory BCoV disease. Advanced age and comorbidities (diabetes, hypertension, heart disease, etc.) are risk factors for severe disease due to SARS, MERS, and COVID-19 (5, 6, 8–10, 40). Various cofactors can exacerbate the severity of BCoV infections and enhance virus transmission or host susceptibility. They include underlying disease or respiratory coinfections, dose and route of infection, and immunosuppression (corticosteroids) (16, 17, 22, 23). Shipping cattle long distances to feedlots and comingling of cattle from multiple sources create physical stresses that can overwhelm the animals' defense mechanisms. These conditions also provide close contact for exposure to high concentrations of new pathogens or strains. Analogous examples for SARS and SARS-CoV-2 are the stress of long airplane trips with close contact among individuals from diverse countries, which may play a role in enhancing an individual's susceptibility or viral transmission (41). Stress-induced corticosteroids cause immunosuppression that reduces the numbers of CD4 and CD8 T cells and certain cytokine levels (42, 43). A recrudescence of BCoV fecal shedding was observed in 1 of 4 WD BCoV-infected cows treated with dexamethasone (31). The BRDC can be precipitated by several viruses, alone or in combination (BCoV, bovine respiratory syncytial virus, parainfluenza-3 virus, bovine herpesvirus) and immunosuppressive viruses (bovine viral diarrhea virus, etc.) (16, 17, 22). For the BRDC, various predisposing factors (viruses, stress) allow commensal bacteria of the nasal cavity (*Mannheimia haemolytica*, *Pasteurella* sp., *Mycoplasma* sp., etc.) to infect the lungs, leading to a fatal fibrinous pneumonia (23) similar to that seen in SARS and COVID-19 patients (40, 44). Bacterial coinfections and their role in the severity of respiratory disease are often overlooked during large outbreaks of human respiratory viral infections. Bacteria have been isolated from SARS cases (*Chlamydia* spp., etc.) (45) and analyzed only in limited studies in SARS-CoV-2 patients (40), but their role in enhancing the severity of SARS-CoV-2 is undefined. Interestingly, in the latter study, 50% of patients with COVID-19

who died had secondary bacterial infections, although most were treated with antibiotics. Antibiotic treatment of animals or SARS or SARS-CoV-2-infected patients coinfected with CoV and bacteria could precipitate massive release of bacterial lipopolysaccharides (LPS). Studies of bovine cells suggest that alveolar macrophages exposed to LPS may orchestrate proinflammatory responses in the lung leading to lung damage (46). Neutrophils recruited by these proinflammatory cytokines can release neutrophil extracellular traps, propagating the inflammation and contributing to adult respiratory distress syndrome and microvascular thrombosis evident in some SARS-CoV-2 patients (47). Recent data showing that altered respiratory microbiota (dysbiosis) is associated with development of BRDC are emerging (48). The influence of the respiratory tract microbiota on COVID-19 severity has not been explored.

DIAGNOSIS OF BOVINE CoV INFECTIONS

As highlighted in the prior sections, BCoVs from cases of diarrhea, winter dysentery, and respiratory disease in cattle and wild ruminants are biologically, genetically, and antigenically similar and comprise a single serotype. Accordingly, BCoV diagnostic reagents should be universally applicable for diagnosis of these clinically distinct syndromes (49). BCoV commonly infects both the respiratory and intestinal tracts with shedding in nasal secretions and/or feces. As with SARS-CoV-2 detection, the sensitivity of the assay and the respiratory specimen used to detect BCoV shedding influence both the detection rates and the length of time the virus is detected. BCoV infections are diagnosed by detection of virus, viral antigen, or viral RNA in tissues, secretions, or excretions of infected animals (reviewed in reference 49). Immunofluorescent (IF) or immunohistochemical (IHC) staining using hyperimmune antiserum or monoclonal antibodies (MAbs) to BCoV is used to detect viral antigen in respiratory (trachea, lung) or intestinal (ileum, colon) tissues (frozen or paraffin-embedded) (23, 49). Although the sensitivity is relatively low, detection of BCoV in nasal secretions or feces by immune electron microscopy (IEM) has the advantage of detecting other viruses as well (28, 29). Bovine CoV antigens are most commonly detected by enzyme-linked immunosorbent assay (ELISA) using a pool of BCoV S and N MAbs to improve assay specificity and sensitivity (16, 17, 22, 49). The ELISA provides rapid test results and is applicable to large numbers of samples. Highly sensitive molecular assays to detect BCoV RNA in nasal secretions, bronchoalveolar lavage (BAL) fluid, lung lysates, or feces are widely used and include RT-PCR, nested RT-PCR, and real-time qPCR assays (26, 31, 49). Importantly these assays should target conserved regions of the BCoV genome (polymerase or N protein) to detect divergent strains. Especially for feces, proper controls are essential to detect interference by PCR inhibitors. In comparisons of nasal swabs (NS), nasopharyngeal swabs (NPS), BAL fluid, and transtracheal wash (TTW) from calves, BCoV was detected by RT-PCR in 15.6%, 20.9%, 14.3%, and 6.6% of NS, NPS, BAL fluid, and TTW samples, respectively (50). Also applicable to SARS-CoV-2, BCoV antigens can be detected directly by IF in nasal epithelial cells collected from nasal swab specimens of BoCV-infected cattle (28, 29). Unlike detection of viral RNA in secretions, antigen detection within infected cells provides direct evidence of BCoV infection of the upper respiratory tract. Notably, the human rectal tumor cell line HRT-18 has been the most efficient to isolate BCoV from feces, nasal swabs, or respiratory tissues of cattle with respiratory disease; nevertheless, some BCoV strains may fail to grow in cell culture (49).

Antibodies to BCoV are quantitated by virus neutralization and hemagglutination inhibition (HI) tests that measure functional neutralizing or hemagglutinating antibodies, respectively, which often correlate with immunity (16, 17, 21, 22, 39, 51, 52). Antibodies to all BCoV strains tested cross-reacted with the classical Mebus strain although some strains had severalfold-higher virus-neutralizing (VN) antibody titers against the homologous strain (16, 17, 21, 22, 51). ELISAs are used to quantitate overall or isotype-specific antibodies (IgM, IgA, IgG1, IgG2) in serum, nasal secretions, or feces, because certain isotypes (i.e., IgA, IgG1, IgG2) may be better correlated with mucosal immunity or neutralizing or HI antibodies (16, 17, 22, 28, 29, 39). Of relevance to SARS-CoV-2, acute-phase serum samples collected only 3 to 4 days after disease onset

did not show unequivocal antibody increases, unless isotype-specific antibody ELISAs were used to detect increases in IgM and IgA antibody titers to BCoV (16, 17, 22, 28, 29, 39). Because BCoV antibodies are widespread in cattle, serologic diagnosis of BCoV infections requires paired acute- and convalescent-phase serum samples.

BCoV INTERSPECIES TRANSMISSION AND WILDLIFE RESERVOIRS

It is now recognized based on analysis of genetic sequences that the emerging human CoVs (SARS, MERS, SARS-CoV-2) from the past 2 decades are zoonoses originating from ancestral bat CoVs (2, 4, 7). SARS and MERS most likely were transmitted from bats to the intermediate animal hosts, civet cats and camels, respectively, and then introduced into humans (2, 5). It is likely that COVID-19 also may have been transmitted from an unidentified intermediate animal host to humans. Thus, interspecies transmission via wildlife and livestock host animals is a key factor in the emergence of these devastating CoVs in humans. We isolated CoVs closely related biologically, antigenically (cross-neutralizing), and genetically to BCoV from captive wild ruminants from the United States including Sambar deer (*Cervus unicolor*), white-tailed deer (*Odocoileus virginianus*), waterbuck (*Kobus ellipsiprymnus*), elk (*Cervus elaphus*), and giraffe (16, 17, 21, 22, 51). Furthermore, serologic studies confirmed the circulation of CoVs that are antigenically closely related to BCoV in native wild ruminants including white-tailed deer, mule deer (51), and caribou (*Rangifer tarandus*) (22). Unfortunately, despite ruminants (camels) being a reservoir host for MERS, few serologic surveys of wild ruminants in native habitats have been done. One of the earliest reports (1995) documenting the interspecies transmission of CoVs with spillover from wildlife reservoirs was the demonstration that CoVs from captive wild ruminants could experimentally infect calves (51). Notably, wild ruminant CoV isolates from Sambar and white-tailed deer and waterbuck infected the upper respiratory and intestinal tracts of gnotobiotic calves and caused diarrhea, and the calves seroconverted with neutralizing antibodies to BCoV (51). Thus, wild ruminants can transmit bovine-like CoVs to cattle or vice versa. In follow-up studies, we sequenced the complete genomes of the CoVs from wild ruminants to assess their genetic similarity to BCoV (53, 54). The giraffe, Sambar and white-tailed deer, waterbuck, and sable antelope CoVs all shared high (99.3% to 99.6%) amino acid sequence identity with enteric and respiratory BCoV strains, supporting their classification as a single species within the BCoV subgenus *Embecovirus*. The above information is directly relevant to SARS, COVID-19, and MERS with spillover of CoVs from wildlife (bats) and ruminants (camels), respectively, to humans.

A common feature of SARS, MERS, and SARS-CoV-2 betaCoVs that is shared with the BCoV betaCoV is that they are promiscuous (5, 16, 17, 22, 51, 53, 54). For largely unexplained reasons, they all have a broad host range and propensity to infect multiple species. Besides detection in wild ruminants, bovine-like CoVs were also identified in other livestock species: water buffalo calves (52) and camelids (alpacas, llamas, and dromedary camels) (55). Another example is the discovery of genetically (>95% nucleotide [nt] identity) and/or antigenically similar CoVs from respiratory samples of dogs with respiratory disease (56). An enteric BCoV also experimentally infected dogs, causing subclinical infection and seroconversion (57). These findings are highly relevant to SARS-CoV-2 because of reports of its transmission from humans to dogs in SARS-CoV-2-infected households (58) and the possibility of its persistence in the susceptible animal host. A concern is that such interspecies infections may establish a host reservoir community and culminate in more genetically divergent CoV strains, including recombinants, increasing the possibility for their transmission to other species.

Notably, 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 (59). An intriguing question is whether dogs or wild birds (such as wild turkeys) could 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. Experimental evidence for interspecies transmission of bovine-like CoVs

between wild ruminants, dogs, birds, and cattle is of concern for open cattle feedlots where wild birds may congregate or cattle may be exposed to dogs, wild ruminants, or their feces.

Highly relevant to COVID-19 as a zoonosis and the zoonotic spillover of MERS CoV from camels (ruminants) to humans is the observation that the common cold human OC43 CoV likely represents an earlier zoonotic transmission of BCoV to humans based on their close genetic and antigenic relatedness (2, 60). The time of the estimated spillover event based on molecular clock analysis was around 1898. It is further projected that a camelid was the intermediate host for human CoV 229E that was introduced to humans around 1718 to 1818 (2, 61). More intriguing was the discovery of a human enteric CoV isolated from a child with acute diarrhea (HECoV-4408) that was genetically (99% nt identity in the S and HE gene) and antigenically more closely related to BCoV than to HCoV-OC43, suggesting that this isolate is a BCoV variant that infects humans (62). We further showed that the HECoV-4408 strain infects the upper respiratory tract and intestine of gnotobiotic calves and causes diarrhea and intestinal lesions (63). It also induces complete cross-protective immunity against the virulent BCoV-DB2 enteric strain, confirming the close similarity of this strain to BCoV (63). The reasons for the broad host range of BCoV are unknown but may relate to the presence of a hemagglutinin and the binding of BCoV to acetylated neuraminic acid, both of which may increase its binding to diverse cell types.

RESPIRATORY CoV VACCINES AND IMMUNITY

No respiratory vaccines have been developed for prevention of PRCV infection of swine, because of its perceived limited economic impact. Although of high economic impact, especially regarding the BRDC, no respiratory BCoV vaccines have been developed to prevent BCoV-associated pneumonia in calves or in cattle with BRDC. The correlates of immunity to respiratory BCoV infections are unclear. However, data from epidemiologic studies of BCoV infections in feedlot cattle show that serum antibody titers to BCoV may be a marker for respiratory protection. In multiple studies, antibody isotype (IgG_1, IgG_2, IgA), neutralizing antibody titer, and magnitude of antibody titer in serum of naturally infected calves or in cattle at arrival in feedlots were correlated with protection against respiratory disease, pneumonia, or BCoV respiratory shedding (16, 17, 22, 29, 39). In one study, intranasal vaccination of calves entering feedlots with a modified live enteric BCoV calf vaccine (licensed for oral use to prevent BCoV diarrhea) reduced the risk of the BRDC in calves (64). Alternatively, if serum BCoV-neutralizing antibodies are a correlate of immunity to respiratory BCoV infection, then parenteral vaccines effective at boosting the low levels of existing BCoV antibodies (most cattle are seropositive for BCoV) may be protective. Vaccines for mucosal pathogens that infect epithelial cells in the respiratory and/or intestinal tracts will likely fail to induce sterilizing immunity needed to prevent respiratory tract reinfections, as observed for natural (28, 29) or experimental (16, 17, 22, 26) respiratory BCoV infections. Consequently, the initial major vaccine focus should be to prevent pneumonia and severe disease.

The correlates of immunity to COVID-19 in humans are also unknown. Mucosal immune responses may be important, particularly to reduce nasal shedding, but mucosal immunity is often short-lived, requiring multiple booster vaccine doses, especially in naive vaccine recipients. A possible scenario as noted above is that SARS-CoV-2 vaccines may prevent severe disease and deaths but may not eliminate nasal shedding, allowing continued transmission (65). Vaccine strategies may need to be altered if SARS-CoV-2 consistently or in certain age groups infects both the respiratory and intestinal tracts (pneumoenteric like BCoV) and is also shed in feces. Oronasal attenuated CoV vaccine prime and parental S vaccine booster may be optimal to prevent both enteric and respiratory infections and fecal and nasal shedding and broaden the CoV immune response as used for some animal CoV vaccines (1).

PRCV IS A NATURALLY OCCURRING RESPIRATORY VARIANT OF TGEV

TGE was first described in the United States in 1946. It is a highly enteropathogenic CoV that causes acute diarrhea and/or vomiting, dehydration, and high mortality in seronegative neonatal piglets (1). PRCV, a naturally occurring respiratory deletion mutant of TGEV with deletions in the S protein, was first isolated in Belgium in 1984 (66). It causes mild respiratory disease, such as coughing, but no enteric disease like the parental TGEV. Compared with TGEV, the PRCV genome contains a large deletion (621 to 681 nt) near the N terminus of the S gene, producing a smaller S protein, and it has variable deletions that compromise ORF3 downstream of the S gene (1). These genetic changes may account for the altered tissue tropism of PRCV (from intestinal to respiratory tract) and its limited intestinal replication (1, 67, 68). Interestingly the S deletion region in PRCV does not affect the receptor binding domain (RBD) or its ability to bind like TGEV to the same host aminopeptidase N (APN) receptor present in the gut and respiratory tract. However, the S deletion in PRCV renders it unable to bind to sialic acids thought to play an essential role in TGEV binding to intestinal mucins and gut infection (69). Investigation of mechanisms whereby changes in the TGEV and PRCV S proteins and ORF3 contribute to alterations of tissue tropisms should contribute to a better understanding of related determinants for other CoVs. Since the emergence of PRCV, the spread of TGEV has also been reduced in PRCV-seropositive herds due to cross-protective immunity with TGEV (1). Therefore, cross-protective immunity between TGEV and PRCV is also an intriguing scenario to investigate the role of CoV S protein in induction of cross-protective VN antibodies between the prototype CoVs and their S gene variants and the contributions of enteric versus respiratory mucosal immunity to local protection (70).

LABORATORY DIAGNOSTIC METHODS FOR PRCV

Laboratory diagnosis of PRCV is accomplished by one or more of the following procedures: detection of viral antigen or nucleic acids in nasal swabs or lesions, virus isolation from respiratory specimens, or detection of PRCV antibodies. An ELISA using monoclonal or polyclonal antibodies to TGEV was used to detect PRCV antigen in cell culture or nasal swabs (71). IF or IHC staining using MAbs to TGEV (nucleocapsid or S protein) was also used to detect PRCV antigen in formalin-fixed, paraffin-embedded lung tissues (42, 72, 73). RT–PCR was used for diagnosis of PRCV and differentiation of PRCV and TGEV (74). Differentiation of PRCV and TGEV was accomplished using PCR primers targeting the S gene deletion region in PRCV strains. Pig kidney and swine testicle cells were used to isolate PRCV from nasal swab fluids or lung tissue homogenates and propagate cell culture-isolated PRCV (1). PRCV serology is complicated due to the cross-reactivity with TGEV (1). Blocking ELISAs differentiated between PRCV and TGEV antibodies based on using monoclonal antibodies to TGEV antigenic sites that are absent on the PRCV S protein (75). However, blocking ELISAs should be applied only on a herd basis because some pigs with low TGEV or PRCV antibody titers might not be detected and the accuracy of commercial ELISAs for differentiating PRCV and TGEV was low (75, 76).

SIMILARITY IN TISSUE OR CELLULAR RESPIRATORY TROPISM OF PRCV TO THAT OF SARS-CoV-2 OR SARS-CoV

Pathogenic features of SARS-CoV and its tropism for the upper and lower respiratory tract. SARS-CoV has a tropism for both the upper and lower respiratory tract. However, SARS-CoV mainly caused severe lower respiratory tract disease (44). Compared with SARS-CoV-2, SARS-CoV showed limited capacity to infect ciliated epithelial cells lining the nasal and bronchial mucosal epithelium based on studies of *ex vivo* cultures of human bronchus and lung and in a nonhuman primate (cynomolgus macaque) model (35, 77). In the infected lower respiratory tract, the infection was characterized by acute damage of alveolar and bronchiolar epithelial cells, especially type 1 and 2 pneumocytes, followed by proliferative and fibrous pneumonia, and pulmonary or multiorgan tissue damage due to immunopathology caused by activated

inflammatory leukocytes and leukocyte-derived cytokines, particularly IFN-α, IL-6, and IL-8 (78, 79), within the pulmonary lesions or in the blood of patients (44) (Table 2). Viral antigens and/or RNA was also identified in the lymphoid organs (lymphocyte depletion), liver, gastrointestinal tract and feces, kidney (tubular necrosis) and urine, central nervous system (degeneration of neurons), and bone marrow (hemophagocytosis) (44).

Pathogenic features of SARS-CoV-2 and its tropism for the upper and lower respiratory tract and potentially the gastrointestinal tract. The pathogenesis of SARS-CoV-2 is poorly understood. Based on multiple similar clinical features, such as fever, atypical pneumonia, acute respiratory distress syndrome (ARDS), and multiple organ failure, especially the cardiovascular system (8, 9), SARS-CoV-2 likely possesses most of the pathogenic or immunopathologic features of SARS-CoV. SARS-CoV-2 has a tropism for both the upper and lower respiratory tract. However, compared with SARS-CoV, SARS-CoV-2 has the capacity to more effectively infect ciliated epithelial cells lining the nasal and bronchial mucosal epithelium based on studies of *ex vivo* cultures of human bronchus and lung and in a nonhuman primate (cynomolgus macaque) model (35, 77) (Table 2). The increased tissue tropism of SARS-CoV-2 to the nasal mucosa may lead to more efficient virus shedding in nasal secretions and person-to-person direct contact or aerosol transmission, compared with SARS-CoV. The following details are based on comparison studies of MERS-CoV, SARS-CoV, and SARS-CoV-2 infections in a nonhuman primate model (77). In the infected lower respiratory tract, similar to SARS-CoV, SARS-CoV-2 also has the capacity to infect type 1 and 2 pneumocytes (77). The latter cells secrete surfactant to reduce surface tension in lung, allowing reinflation of the alveoli following exhalation. SARS-CoV-2 causes diffuse alveolar damage (DAD) with exudates as a result of extensive destruction of type 1 pneumocytes lining the alveoli (77), followed by type 2 pneumocyte hyperplasia and hypertrophy and infiltration of neutrophils, macrophages, and/or lymphocytes, causing thickened alveolar septa, pulmonary fibrosis, and increased inflammatory responses (8, 77). In addition to pulmonary damage, similar to SARS-CoV (78, 79), SARS-CoV-2 infection may also be directly or indirectly responsible for multiple organ failure or multiorgan tissue damage, especially the cardiovascular system, due to hypoxia and immunopathology caused by activated inflammatory leukocytes and leukocyte-derived cytokines in the blood of patients (8, 40). In SARS-CoV-2 patients, there is also evidence for viral RNA in the gastrointestinal tract and feces (as with SARS-CoV cases [19]) and central nervous system (cerebrospinal fluid) (20, 80).

Pathogenic features of PRCV and its tissue tropism for the upper and lower respiratory tract. Similar to SARS-CoV-2 or SARS-CoV, PRCV has a tropism for both the upper and lower respiratory tract. Occasionally, PRCV was also detected in the small intestines, tracheobronchial lymph nodes, and blood of infected pigs (1, 67). However, PRCV did not replicate efficiently in villous enterocytes, with only low viral titers in intestinal contents (1, 67). PRCV replicated to moderate to high titers in lungs (up to $10^{8.3}$ 50% tissue culture infective doses [$TCID_{50}$]/g) at 4 to 8 days postinoculation (dpi) (67), accompanied by moderate to marked consolidation (42, 73, 81). In the infected upper and lower respiratory tract, PRCV antigens were found in type 1 and 2 pneumocytes and, to a lesser extent, epithelial cells of the nares, trachea, bronchi, bronchioles, and occasionally alveolar macrophages (42, 67, 72) (Table 2). PRCV causes bronchoalveolitis as a result of necrosis of epithelial cells lining the upper and lower respiratory tract, followed by type 2 pneumocyte hyperplasia and hypertrophy and infiltration of macrophages and lymphocytes, causing thickened alveolar septa (42, 67, 72) and increased inflammatory responses, including IFN-α, TNF-α, IL-6, IFN-γ, and IL-12 in lung (72, 73, 82), similar to SARS-CoV-2 or SARS-CoV patients (8, 40, 78, 79). Increased innate cytokines that occur early in the lung of PRCV-infected pigs may inhibit initial viral replication and modulate Th1/Th2 responses with the latter enhancing B-cell responses, effectively leading to secretion of VN antibodies. Nasal virus shedding lasted for 9 to 10 dpi, with peak viral titers at 1 to 2 dpi (42, 72, 73). The severity of pneumonia and viral replication in lung peaked at 7 to 8 dpi and then resolved concurrently with increased VN antibody titers (42, 72, 73).

Some pathogenic features of PRCV distinct from SARS-CoV-2 or SARS-CoV. PRCV-related respiratory disease is mostly mild and self-limiting unless complicated by other factors reviewed in the next section. Like PRCV in pigs, most SARS-CoV-2- or SARS-CoV-infected individuals have mild or subclinical disease and recover. In severely affected patients, the respiratory disease has the potential to be irreversible and may be complicated by a cytokine storm and multiple organ failure. In a nonhuman primate model, SARS-CoV-2 or SARS-CoV caused severe DAD and pulmonary edema as a result of extensive destruction of type 1 pneumocytes lining the alveoli (77), whereas in pigs PRCV causes only bronchoalveolitis and airway plugging characterized by mild to moderate accumulation of necrotic cells and inflammatory cells in the bronchial and bronchioalveolar lumens (42, 67, 72) (Table 2). Like SARS-CoV, severe SARS-CoV-2 infections are also frequently associated with multiple organ failure or multiorgan tissue damage due to immunopathology caused by dysregulated and increased proinflammatory systemic immune responses in patients (8, 40). In comparison, there is much less systemic proinflammatory cytokine responses in PRCV-infected pigs (43), consistent with mild or subclinical disease. In addition, unlike PRCV infection, neutrophils frequently infiltrate at the infection sites in the lung or in the blood (i.e., neutrophilia) of COVID-19 patients, although the related mechanisms are unclear (47). One of the by-products released from neutrophils, neutrophil extracellular traps (NETs), are involved with formation and progression of the pulmonary embolism or thrombosis, ARDS, etc. (47). The exact role of neutrophils and NETs in COVID-19 should be defined.

PIG MODEL OF FACTORS THAT ENHANCE PRCV ACUTE RESPIRATORY INFECTIONS AND DISEASE

Bacteria. Acute respiratory CoV-bacterium-mediated respiratory disease was reproduced in PRCV-infected pigs cotreated with lipopolysaccharides (LPS) from a Gram-negative bacterium or lipoteichoic acids (LTA) from a Gram-positive bacterium (82–84). At 24 h after intratracheal PRCV inoculation, pigs were inoculated intratracheally with low doses of *Escherichia coli* (O111:B4) LPS (20 μg/kg of body weight) or *Staphylococcus aureus* LTA (200 μg/kg) (82–84). All pigs treated with low doses of either LPS or LTA alone recovered from clinical signs, such as anorexia, for at least $<$8 h after LPS or LTA inoculation, but they showed increased proinflammatory responses in the lungs at 4 to 8 h after LPS or LTA inoculation. PRCV and LPS or LTA cotreated pigs showed greater respiratory disease compared with pigs treated with either PRCV alone or LPS or LTA alone (82, 84), indicating synergistic interactions between PRCV and LPS or LTA. The PRCV pig model was used in a previous study (83), to investigate if an anti-TNF-α drug, etanercept (Embrel), reduces the severity of clinical disease, lung lesions, virus replication in lungs, and lung cell infiltration or levels of IFN-α, IL-1, IL-6, and IL-12/IL-23 in PRCV and LPS cotreated pigs compared with the etanercept-untreated counterparts. No effect of etanercept was evident.

Respiratory viral coinfections. Dual infections of pigs with the arterivirus (order *Nidovirales*, like CoV) porcine reproductive and respiratory syndrome virus (PRRSV) followed by PRCV resulted in severe pneumonia with increased PRCV (antigen), prolonged fever with respiratory disease, and reduced weight gain compared with each virus alone (73, 85). Ongoing or preexisting PRRSV infection significantly suppressed innate immune responses (reduced IFN-α levels in lung and blood natural killer cell cytotoxicity) during early PRCV infection, which may exacerbate PRCV pneumonia (73). In another dual viral infection model (highly relevant to potential SARS-CoV-2 and influenza virus coinfections), pigs were first inoculated with PRCV followed in 2 to 3 days by swine influenza A virus (SIV) (86). SIV lung titers were reduced in the dually infected pigs compared with the singly infected pigs, but the lung lesions were more severe in the dually infected pigs. The high levels of IFN-α induced by PRCV may have mediated interference with SIV replication but also may have contributed to the enhanced lung lesions. These studies are also relevant to potential treatments of COVID-19 patients with IFN-α.

Immunosuppression (corticosteroids). The practical effectiveness of corticosteroid therapy for COVID-19 patients needs to be studied further. During the previous SARS-CoV outbreaks, the use of corticosteroids in SARS patients caused significant adverse effects, including secondary viral/fungal/bacterial infections due to the immunosuppressive effects and steroid-induced avascular necrosis and myopathy (87). Pigs were administered intramuscularly (i.m.) the corticosteroid dexamethasone (DEX) for 6 days and immunosuppressed, followed by PRCV inoculation (42). DEX treatment alleviated initial PRCV pneumonia at 2 dpi but exacerbated later stages of infection (4 to 21 dpi), possibly by decreasing cellular immune responses in the lungs (IFN-γ-secreting T cells), thereby creating an environment for more-extensive CoV replication. These data have potential implications for corticosteroid use with COVID-19 patients and suggest a precaution against prolonged use.

CONCLUSIONS

Although BCoV is a betaCoV (subgenus *Embecovirus*) like SARS-CoV and SARS-CoV-2 (subgenus *Sarbecovirus*) and PRCV is an alphaCoV, both animal respiratory CoVs share some common features with SARS-CoV and SARS-CoV-2. BCoV has a broad host range including wild ruminants and a zoonotic potential. BCoV also has a dual tropism for the respiratory and gastrointestinal tracts. Other shared aspects include an array of clinical symptoms and syndromes in the host (depending on age, coinfections, and stress), the patterns of respiratory disease, lung lesions, and a potential for recurrent nasal shedding. PRCV has a tissue tropism for the upper and lower respiratory tracts and a cellular tropism for type 1 and 2 pneumocytes in lung but is generally a mild infection unless other exacerbating factors ensue, such as bacterial or viral coinfections and immunosuppression (corticosteroids). An understanding of animal CoV infections in the natural host is critical to provide a One Health perspective and insights on common and distinctive disease mechanisms related to SARS-CoV-2 infection and the potential viral or host factors that contribute to the severity of COVID-19. Lastly, PRCV and LPS or LTA cotreated pigs are also a useful biosafety level 2 (BSL2) animal model to study acute respiratory CoV-bacterium-mediated respiratory disease, including COVID-19, and to test therapeutics such as immunomodulators designed to control immunopathology in the lungs or blood of COVID-19 patients.

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