Chapter 18

Coronaviruses of Domestic Livestock and Poultry: Interspecies Transmission, Pathogenesis, and Immunity

Linda J. Saif

In 2002–2003, severe acute respiratory syndrome (SARS), a new fatal respiratory disease of humans, appeared in China and rapidly spread globally (24, 55, 76, 77, 83). It was caused by a previously unrecognized coronavirus (CoV), SARS-CoV, that was likely of zoonotic origin from a wildlife reservoir (bats or civet cats) (33, 58, 62). The emergence of SARS-CoV stunned the medical community, but animal coronavirologists had previously documented the propensity of CoVs to cause fatal respiratory and enteric disease in animals, their interspecies transmission, and the existence of wildlife reservoirs (90, 91). Although there was compelling evidence for emergence of new CoV strains and genetic changes in existing strains leading to new tissue tropisms or disease syndromes in animals, the extensive diversity and disease impact of CoVs were not widely appreciated before the SARS epidemic.

Like SARS-CoV, the CoVs of domestic livestock and poultry cause primarily respiratory and/or enteric disease (Table 1). These animal CoVs belong to each of the three established CoV groups, with two subgroups recognized for groups 1 (1a and 1b) and 2 (2a and 2b) (Table 1). The swine enteric CoVs, porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV), and the spike protein (S protein) gene deletion respiratory mutant of TGEV, the porcine respiratory CoV (PRCV), belong to group 1. The latter two CoVs (group 1a) are closely related genetically (98 and 97.6% nucleotide identity between the complete genomes of PRCV ISU-1 and Miller and Purdue TGEV strains, respectively) (122) and antigenically (60, 95), with the major immunodominant neutralizing antigenic site (A) conserved on PRCV and TGEV. PEDV is more distantly related and is in subgroup 1b with human CoVs 229E and NL63 and bat CoV. Antibodies to PEDV do not neutralize TGEV or PRCV, although a shared antigen was shown by immunoblotting analysis (80). A further unexplained observation is the antigenic cross-reactivity between the group 1a CoVs and SARS CoV, likely at the level of the N protein (34a, 55, 105).

Bovine CoV (BCoV) (which causes pneumoenteric infections in cattle), wild-ruminant CoVs, and hemagglutinating encephalomyelitis virus swine (HEV) belong to CoV subgroup 2a along with canine respiratory CoV and human CoVs OC43 and HKU1. With the exception of HEV, which also infects the central nervous system, causing a wasting disease (79), the viruses cause enteric and/or respiratory disease (Table 1). Similarly, the recently discovered SARS-CoVs that are associated with both respiratory and enteric infections in humans and animals (civet cats, raccoon dogs, and bats) belong to a new CoV subgroup, 2b. An important difference between group 1 and 3 CoVs and group 2 CoVs is the presence of a hemagglutinin-esterase (HE) glycoprotein in a subset of group 2 CoVs, including BCoV and wild-ruminant CoVs. HE forms a second dense layer of short surface projections, in contrast to the longer surface S glycoproteins present on all CoVs, as illustrated in Fig. 1.

Avian CoVs are the exclusive members of CoV group 3. They cause respiratory and enteric infections, but some strains of the chicken CoV, infectious bronchitis virus (IBV), also cause nephritis and infections of the reproductive tract (10, 19).

The focus of this chapter is on representative CoVs of livestock and poultry from each of these three groups, with emphasis on the respiratory and enteric CoV infections and analogies to SARS-CoV.

Linda J. Saif • Food Animal Health Research Program, Ohio Agricultural Research and Development Center, and Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Wooster, OH 44691.

			Disease or infected tissue			
Group	Virus	Host	Respiratory	Enteric ^a	Other	
1a	Transmissible gastrointestinal virus (TGEV)	Pig	X (upper)	X(SI)		
	Porcine respiratory coronavirus (PRCV)	Pig	X (upper/lower)		Viremia	
	Canine enteric CoV (CECoV)	Dog		Х		
	Feline coronavirus (FCoV)	Cat	Х	Х	Systemic (FIPV)	
1b	Human coronaviruses 229E and NL63	Human	X (upper/lower)		-	
	Porcine epidemic diarrhea virus (PEDV)	Pig		X (SI, colon)		
	Bat (Miniopterus) CoV	Bat	X (upper)	Х	Subclinical?	
2a	Human coronaviruses OC43 and HKU1	Human	X (upper)	?? (BCoV?)		
	Hemagglutinating encephalitis virus (HEV)	Pig	X		CNS^{b}	
	Bovine coronavirus (BCoV)	Cattle	X (lung)	X (SI, colon)		
	Wild ruminant CoVs ^c	Multiple	?	Х		
	Canine respiratory CoV (CRCoV)	Dog	Х	?		
2b	Severe acute respiratory syndrome (SARS) CoV	Human	X (lung)	Х	Viremia, kidney?	
	Civet cat (Paguma larvata) CoV	Palm civet	X	Х	Subclinical?	
	Raccoon dog (Nyctereutes procyonoides) CoV	Raccoon dog	?	Х	Subclinical?	
	Horseshoe bat (Rhinolophus sinicus) CoV	Bat	?	Х	Subclinical?	
3	Infectious bronchitis virus (IBV)	Chicken	X (upper)	X (no diarrhea)	Viremia, kidney, oviduct	
	Turkey coronavirus (TCoV)	Turkey		X (SI)		

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^aSI, small intestine; ??, BCoV-like CoV from a child (123).

^bCNS, central nervous system.

^c Wild-ruminant CoVs include isolates from sambar deer (*Cervus unicolor*), white-tailed deer (*Odocoileus virginianus*), waterbuck (*Kobus ellipsiprymnus*) (108), giraffe (*Giraffa camelopardalis*) (36a), and elk (*Cervus elaphus*) (65).

The goal is to provide insights into comparative aspects of transmission, pathogenesis, and immunity for these animal CoVs.

EPIDEMIOLOGY, INTERSPECIES TRANSMISSION, AND WILDLIFE RESERVOIRS

The likelihood that SARS-CoV is a zoonotic infection transmitted from wild animals to humans is not unprecedented based on previous veterinary research on interspecies transmission and wildlife reservoirs for animal CoVs. In this section, the initial descriptions, epidemiology, interspecies transmission, and possible wildlife reservoirs for selected group 1, 2, and 3 animal CoVs are reviewed.

Group 1 CoVs

Subgroup 1a: TGEV and PRCV epidemiology

Transmissible gastroenteritis (TGE) has been recognized in swine since 1946 and occurs worldwide (95). Both epidemic and endemic forms of TGE occur, especially in North America; however, endemic TGEV is more common in Europe and Asia due to the widespread presence in pigs of the respiratory variant PRCV, which induces at least partial immunity to TGEV (95). Epidemic TGEV occurs primarily during winter in TGEV/PRCV-seronegative herds, infecting pigs of all ages but producing severe diarrhea and dehydration in suckling pigs under 2 to 3 weeks of age. Mortality is high in this population (often 100%). Endemic TGEV occurs in partially immune herds, including PRCV-seropositive herds. It is especially common in herds with continuous farrowing due to the presence of susceptible pigs postweaning, after loss of maternal antibodies. Morbidity may be high, but mortality is low (10 to 20%) and related to the age when infected. Interestingly, the seroprevalence of TGEV in Europe has declined significantly coincident with the spread of PRCV. Besides the small intestine, TGEV also infects the upper respiratory tract and is transiently shed nasally; nevertheless, the major transmission route appears to be fecal-oral (53, 117).

PRCV, a mutant of TGEV with S protein gene deletions of various sizes (621 to 681 nucleotides [nt]), altered tissue tropism (respiratory), and reduced virulence, emerged independently in the 1980s in Europe (78) and the United States (118). Smaller deletions also occurred proximal to or in open reading frame 3a (ORF3a) (encoding an undefined non-structural protein), resulting in its lack of expression (54, 60, 75). Based on the S protein gene deletion with loss of an antigenic site (D), TGEV and PRCV strains in clinical specimens (nasal secretions/tissues, gut tissues, or feces) or antibodies in serum can be differentiated genetically by nested reverse transcriptase



Figure 1. Immuno-EM of tissue culture-adapted animal CoVs. Particles were reacted with antisera to TGEV (a) or BCoV (b and c). (a) PRCV showing single layer of surface spikes (arrow); (b and c) group 2 CoVs (WD strain of BCoV [b] and sambar deer CoV [c] showing shorter surface HE (arrowhead) and longer spikes (arrow) resulting in a dense outer fringe. Bar = 100 μ m.

PCR (RT-PCR) assays (54, 74) or antigenically by use of monoclonal antibodies (MAbs) to site D in blocking enzyme-linked immunosorbent assay (ELISA) (95), respectively.

PRCV infections are often subclinical or associated with mild respiratory disease, although lung lesions are almost invariably present. The virus has become endemic in many European swine herds, and PRCV infections also occur in Asia (60, 95). A small serologic survey in the United States of asymptomatic swine herds in Iowa reported that many were seropositive for PRCV antibodies (119). Swine population density, distances between farms, and season influence the epidemiology of PRCV. The virus spreads long distances (several kilometers) by airborne transmission or directly by contact. Although PRCV-seronegative pigs of all ages are susceptible to infection, PRCV persists in closed herds by infecting newly weaned pigs, after passive maternal antibodies have declined. PRCV, like TGEV, can disappear from herds in summer and reemerge in older pigs in winter (60, 78, 95) potentially persisting as subclinical infections in pigs in summer or perhaps in other animal reservoirs (see Interspecies Transmission). It is notable that in Europe (but less so in North America) the more virulent enteric TGEV infections have been displaced following the widespread dissemination of PRCV.

Subgroup 1b: PEDV epidemiology

In the late 1970s through the 1980s a new porcine CoV, PEDV, appeared first in Europe and then throughout Asia (80). However, to date there are no reports of PEDV in North or South America. The initial PEDV outbreaks in Europe resembled TGE, with fecal-oral transmission and severe diarrhea, but with slower spread, lower overall mortality in baby pigs (average, 50%), and more marked variation in morbidity and mortality in breeding herds. Such acute outbreaks with high piglet mortality are now uncommon in Europe, but until recently they accounted for enormous losses (thousands of pigs) among suckling pigs in Asia. Currently PEDV appears to have become endemic in several Asian countries, unlike in some European countries where PEDV outbreaks are rare and the prevalence has declined markedly. However, recent wide-scale serologic surveys are lacking.

Subgroup 1a: TGE/PRCV interspecies transmission and wildlife reservoirs

Interspecies transmission has been documented experimentally for the various subgroup 1a CoVs most closely related to the TGEV/PRCV cluster, including canine enteric CoV (CECoV) and feline CoV (FCoV). These viruses share close biologic, antigenic, and genetic relationships and may represent host range mutants of an ancestral CoV (46, 69, 95). They crossreact in virus neutralization tests and with MAbs to the S, nucleocapsid (N), or membrane (M) protein, and all share antigenic subsite Ac on the S protein. Interestingly, neutralizing immunoglobulin G (IgG) MAbs to the S protein of TGEV effectively mediate antibodydependent enhancement of FCoV (feline infectious peritonitis virus [FIPV] strains) infection of macrophages in vitro, demonstrating the functional crossreactivities of the S antibodies (72). The type II FCoV and CECoV and TGEV share >90% amino acid identities in the M and N proteins and >80% overall amino acid identities in the S protein, with >94% identity from amino acids (aa) 275 to 1447 (31). TGEV, PRCV, and FCoV also share a common receptor, aminopeptidase N. They cross-infect pigs, dogs, and cats with

variable levels of disease expression and cross-protection in the heterologous host (92, 95).

Possible wild and domestic animal reservoirs for TGEV were recognized prior to their postulated role in the SARS-CoV outbreak. Wild and domestic carnivores (foxes, dogs, and, possibly, mink) seroconvert to TGEV positivity and are suggested as potential subclinical carriers of TGEV, serving as reservoirs between seasonal (winter) epidemics, but only virus excreted by dogs has been confirmed as infectious for pigs (95). Wild birds (*Sturnus vulgaris*) and flies (*Musca domestica*) have been proposed as mechanical vectors for TGEV.

Subgroup 1b: PEDV interspecies transmission and wildlife reservoirs

The emergence and origin of PEDV in European swine described in the late 1970s and subsequently in Asia remain an enigma. Less cross-reactivity has been reported between the TGEV-related CoVs and PEDV using polyclonal antisera or MAbs, except for oneway reactivity with the N protein (80, 95). Also, unlike the other animal group 1 CoVs, PEDV grows in Vero cells (African green monkey kidney), as do SARS-CoVs. Of interest, a group 1 CoV was recently discovered in fecal and respiratory samples of bats (*Miniopterus* spp.) in Hong Kong with the nucleotide sequences of the RNA-dependent RNA polymerase and S protein gene fragments most closely related to those of PEDV (82). More full-length genomic sequence data for multiple group 1 CoV isolates, including additional ones from wildlife, may provide further insight into group 1 CoV genetic relationships, origin, and common ancestors.

Group 2 CoVs

Subgroup 2a: BCoV epidemiology

Bovine CoV is a pneumoenteric virus that is shed in both feces and upper respiratory tract secretions. It is ubiquitous in cattle worldwide, based on BCoV antibody seroprevalence data (18, 86, 92). The past three decades have witnessed dramatic breakthroughs in our understanding of the role of BCoV in three distinct clinical syndromes in cattle (Table 2): calf diarrhea (18, 92); winter dysentery (WD)with hemorrhagic diarrhea in adults (4, 13, 86, 92, 107, 109, 110, 114); and respiratory infections in cattle of various ages, including the bovine respiratory disease complex (BRDC) or shipping fever of feedlot cattle (15, 18, 29, 37–39, 42, 43, 56, 57, 102–104, 106).

Calf diarrhea associated with BCoV infection is most common in calves under 3 weeks of age, when passively acquired colostral and milk antibody levels have declined, but clinical disease may occur in calves up to 3 months of age (Table 2) (18, 42, 43, 92). The occurrence of severe diarrhea, resulting in dehydration and death, depends on BCoV dose, calf age, and calf immune status. Enteric infections with BCoV have been reported for calves, with prevalence rates of 8 to 69% in diarrheic calves and 0 to 24% in subclinically infected calves. Mixed infections with other enteric pathogens, such as rotavirus, calicivirus, and *Cryptosporidium*, are common, and the additive effect of multiple pathogens on calf disease severity is also recognized (85). Disease is more prevalent in winter, probably due to greater viral stability in the cold, and outbreaks often occur yearly on the same farm.

BCoV is also implicated as a cause of mild respiratory disease (coughing and rhinitis) or pneumonia in 2- to 6-month-old calves and is detected in nasal secretions, the lungs, and often the intestine and feces (Table 2) (18, 42, 43, 68, 84, 92). In studies of calves from birth to 20 weeks of age, Heckert et al. (42, 43) documented both fecal and nasal shedding of BCoV, but with diarrhea most prominent upon initial infection of calves with BCoV. Subsequently repeated or intermittent respiratory shedding episodes occurred in the same animal, with or without respiratory disease, but with subsequent transient increases in serum antibody titers consistent with these reinfections. These findings further suggest a lack of long-term mucosal immunity in the upper respiratory tract after natural BCoV infection, confirming similar observations for human respiratory CoV (7) and PRCV (6). Consequently, within a herd, reservoirs for BCoV infection may be virus cycling in clinically or subclinically infected calves, young adult cattle in which sporadic nasal shedding prevails, or clinically or subclinically infected adults. BCoV is transmitted via both fecal-oral and potentially respiratory (aerosol) routes.

WD occurs in adult dairy and beef cattle and in captive wild ruminants during the winter months and is characterized by hemorrhagic diarrhea, frequent respiratory signs, anorexia, and a marked reduction in milk production in dairy cattle (Table 2) (13, 86, 92, 110, 114). WD occurs most commonly from November to March in the northern United States. It has also been reported in Europe, Australia, and Asia (86, 92, 114). The morbidity rate ranges from 20 to 100%, but the mortality rate is usually low (1 to 2%), although longer-term reduced milk production has been reported. BCoV has been implicated as a cause of WD both in epidemiological studies (99) and in experimental transmission studies in seropositive nonlactating (110) or seronegative lactating dairy cows (107). Although BCoV is the major etiologic agent associated with WD, other host, environmental, or viral factors and interactions related to disease

Disease symdrome	Clinical signs	Cells infected	Lesions ^a		Shedding ^b		
Disease syndrome			Respiratory	Enteric	Nasal	Fecal	Ages affected
Calf disease syndromes							
Calf diarrhea	Diarrhea Dehydration Fever, anorexia	Intestinal, nasal, ± lung epithelial cells	+/- Lung emphysema	++ J, I, colon Villous atrophy	2–8 days	2–8 days	Birth–4 wk
Calf pneumonia	Cough Rhinitis ± Pneumonia ± Diarrhea Fever, anorexia	Nasal ± lung Tracheal ±Intestinal epithelial cells	+/– Pneumonia	+/– J, I, colon Villous atrophy	5 days	NR	2 wk-6 mo
Young adult/ adult disease syndromes							
WĎ	Hemorrhagic diarrhea Dehydration ±Rhinitis, dry cough Feyer anorevia	Intestinal, nasal ± lung epithelial cells?	NR	++ J, I, colon Enterocolitis	+/-	1–4 days	6 mo–adult
Shipping fever or BRDC	Cough, dyspnea ± Rhinitis ± Pneumonia ± Diarrhea Fever, anorexia	Nasal, trachea Bronchi, alveoli ± Intestinal epithelial cells	Interstitial emphysema Bronchiolitis Alveolitis ± Bacteria	+/- NR	5–10 days (17 days)	4–8 days (17 days)	6–10 mo

Table 2. Summary of disease syndromes associated with DCOV infectio	Table 2.	. Summary	of disease	syndromes	associated	with BCoV	infections
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^{*a*}, J, jejunum; I, ileum; NR, not reported; +/-, mild or no lesions; ++, moderate to severe lesions.

^bShedding detected by infectivity or antigen assays; parentheses denote shedding detected by RT-PCR. In experimental challenge studies, the incubation periods for disease onset and shedding both ranged from 2 to 8 days.

manifestation, especially the severe bloody diarrhea and winter prevalence, remain unknown.

Since 1995, BCoV has been increasingly implicated in the BRDC associated with respiratory disease and reduced growth performance in feedlot cattle (Table 2) (15, 37, 56, 57, 102–104, 106). BCoV was detected from nasal secretions and lungs of cattle with pneumonia and from feces (37, 39, 56, 57, 102– 104, 106). In a subsequent study, a high percentage of feedlot cattle (45%) shed BCoV both nasally and in feces as determined by ELISA (15). Application of nested RT-PCR detected higher BCoV nasal and fecal shedding rates, 84 and 96%, respectively (37). Storz et al. (103) reported that 25 of 26 Texas feedlot cattle that died had a BCoV infection and that both BCoV and *Pasteurella* were isolated from lungs of the cattle with necrotizing pneumonia.

Some investigators have shown an association between nasal shedding of BCoV and respiratory disease. In a large feedlot study (n = 1,074 cattle), Lathrop et al. (56) noted that feedlot calves shedding BCoV nasally and seroconverting to BCoV positivity (>4fold) 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 BCoV. Similarly, in studies by Hasoksuz et al. (37) and Thomas et al. (106), calves shedding BCoV nasally were 2.7 and 1.5 times, respectively, more likely to have respiratory disease than calves that were not shedding. In another study, nasal shedding of BCoV increased the risk of requiring treatment for respiratory disease (81). Intranasal vaccination of such calves using a commercial modified live BCoV calf vaccine on entry to a feedlot reduced the risk of calves developing the BRDC.

From these studies, we conclude that both respiratory and enteric shedding of BCoV are common in feedlot cattle, with peak shedding at 0 to 4 days after arrival at feedlots. In one study, in which 3-dayprearrival specimens were tested, nasal shedding consistently preceded fecal shedding (106). Additionally, many cattle (61 to 74%) shed BCoV at the buyerorder barn prior to shipping to feedlots (104). A high percentage (91 to 95%) of feedlot cattle seroconverted (2 to 4-fold-increased titers) to BCoV positivity by 3 weeks postarrival. An important observation from several studies was that cattle arriving with relatively high BCoV antibody ELISA titers or neutralizing antibodies in serum were less likely to shed BCoV, seroconvert, or develop the BRDC (15, 57, 64, 66, 106). Furthermore, some investigators have shown that BCoV infections had a negative impact on weight gains in feedlot cattle (15, 37), which suggests that

BCoV may have an impact on herd health and performance. Similarly, higher titers in serum of antibody against BCoV have been associated with increased weight gains (66, 106).

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 two or three subtypes identified by neutralization tests or using MAbs (18, 38, 39, 92, 109). Although genetic differences (point mutations but not deletions) have been detected in the S protein gene between enteric and respiratory isolates, including ones from the same animal (16, 40), in vivo studies revealed a high level of cross-protection between such isolates (14, 26). Inoculation of gnotobiotic or colostrum-deprived calves with calf diarrhea, WD, or respiratory BCoV strains led to both nasal and fecal CoV shedding followed by complete cross-protection against diarrhea after challenge with a calf diarrhea strain (14, 26). However, subclinical nasal and fecal virus shedding (detected only by RT-PCR) in calves challenged with the heterologous BCoV strains (14, 26) confirmed field studies suggesting that subclinically infected animals may be a reservoir for BCoV in infected herds (42, 43). Cross-protection against BCoV-induced respiratory disease has not been evaluated.

Subgroup 2a: BCoV interspecies transmission and wildlife reservoirs

The likelihood that SARS-CoV of humans is a zoonotic infection potentially transmitted from wild animals is not surprising in light of the previous identification of possible wildlife reservoirs for BCoV. Captive wild ruminants from the United States, including sambar deer (Cervus unicolor), whitetailed deer (Odocoileus virginianus), waterbucks (Kobus ellipsiprymnus), elk (Cervus elaphus), and, more recently, giraffes (36a), harbor CoVs biologically (growth in HRT-18 cells and hemagglutinin of mouse and chicken erythrocytes) and antigenically (cross-neutralizing) closely related to BCoV (65, 108). The deer and waterbuck isolates were from animals with bloody diarrhea resembling WD in cattle (108). Although CoVs were previously detected by electron microscopy (EM) and ELISA (BCoV antigen specific) in diarrheic feces of sitatungas (Tragelaphus spekei) and musk oxen (Ovibos moschatus) from a wildlife park in England, they failed to replicate in vitro (HRT-18 cells) or in vivo (gnotobiotic calves) (11).

Serologically, 6.6 and 8.7% of sera from whitetailed deer in Ohio and mule deer in Wyoming, respectively, were positive for antibodies to BCoV by indirect immunofluorescence tests (108). Caribou (*Rangifer tarandus*) were also BCoV seropositive (25). These studies confirm the existence of CoVs in captive and native wild ruminants that are antigenically closely related to BCoV. Thus, the possibility exists that native wild ruminants may transmit bovine-like CoVs to cattle (see below) or visa versa. Unfortunately, few serologic surveys of wild ruminants in native habitats have been done. Moreover, most of the CoVs from wild ruminants have not yet been sequenced to assess their genetic similarity to BCoV. An exception is the recent full-length genomic sequence for an antigenically related giraffe CoV (GiCoV-OH3) that shares 99.3 to 99.6% amino acid identity with two enteric BCoV strains (36a).

Although many CoVs have restricted host ranges, some, such as the subgroup 2a BCoV and, more recently, the subgroup 2b SARS-CoV, appear to be promiscuous (90, 91). Coronaviruses genetically (>95% nucleotide identity) and/or antigenically similar to BCoV have been detected from respiratory samples of dogs with respiratory disease (27) and also from humans (123) and wild ruminants (see prior section). A human enteric CoV isolate from a child with acute diarrhea (HECoV-4408) was genetically (99% nucleotide identity in the S protein and HE genes with BCoV) and antigenically more closely related to BCoV than to human CoV OC43, suggesting that this isolate is a BCoV variant. More evidence for this likelihood was the recent report that the HECoV-4408 strain infects (upper respiratory tract and intestine) and causes diarrhea and intestinal lesions in gnotobiotic calves (36). It also induces complete cross-protective immunity against the virulent BCoV-DB2 enteric strain. Notably, the wild-ruminant CoV isolates from sambar and white-tailed deer, giraffe, and waterbuck also infected the upper respiratory and intestinal tracts of gnotobiotic calves and caused diarrhea (36a, 108), affirming experimentally that wild ruminants may serve as a reservoir for CoV strains transmissible to cattle.

Bovine CoV can also experimentally infect and cause mild disease (diarrhea) in phylogenetically diverse species such as avian hosts, including baby turkeys but not baby chickens (48). It is notable that in the latter study, the BCoV-DB2 calf-virulent strain infected baby turkeys, causing diarrhea and reduced weight gain, and the virus was transmitted to unexposed contact control birds. However, in an earlier study (22) using cell culture-adapted BCoV strains as an inoculum in turkey poults, intestinal infectivity was seen but without lesions or clinical disease, suggesting that BCoV strain or virulence differences (after cell culture adaptation) can influence BCoV pathogenicity for turkey poults. These data raise intriguing questions of whether wild birds (such as wild turkeys) could also be a reservoir for bovinelike CoVs transmissible to cattle or wild ruminants or, conversely, if cattle (or ruminants) can transmit CoVs to 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 (25, 108). The reasons for the broad host range of BCoV are unknown but may relate to the use of acetylated neuraminic acid as a host cell receptor or the presence of a hemagglutinin on BCoV. Both factors may have a role in binding to diverse cell types.

Group 3 CoVs

IBV and TCoV epidemiology

Infectious bronchitis is a highly contagious, ubiquitous respiratory disease of chickens that is endemic worldwide (10, 19). It is caused by the CoV IBV, first isolated in the 1930s. Like SARS-CoV, it is spread by aerosol or, possibly, fecal-oral transmission. Genetically and antigenically closely related CoVs have been isolated from pheasants and turkeys (9, 10, 32, 34, 47), but in young turkeys, turkey CoV (TCoV) causes mainly enteritis. Recently, other galliform birds (peafowl, guinea fowl, and partridge) were found to be infected by CoVs genetically similar, if not identical, to IBV strains (9). CoVs representing potential new species within group 3, based on partial genomic sequence data, were identified from a graylag goose (Anser anser), a mallard duck (Anas platyrhynchos), and a pigeon (Columbia livia) using pan-CoV RT-PCR (9). Unfortunately, these CoVs could not be isolated by inoculation into embryonated domestic fowl eggs for further studies.

IBV is heterogeneous, having multiple serotypes with extensive antigenic variation and broad tissue tropisms and pathogenicity. IBV strains differ in their virulence, but the host genetic background can also influence infection outcome (2, 9, 19). Respiratory infections of chickens with IBV are characterized by tracheal rales, coughing, and snicking (sneezing), but not pneumonia. The disease is most severe in chicks (10, 19). IBV also replicates in the oviduct, causing decreased egg production or quality. Nephropathogenic strains infect the kidneys, causing mortality in young birds, whereas in broilers, death ensues from systemic Escherichia coli infections after IBV damage to the respiratory tract. Interestingly, the genetically closely related pheasant CoV (PhCoV) causes disease (respiratory and kidney) similar to that of IBV in chickens (9, 32).

TCoV has been identified in North America, Australia, the United Kingdom, Italy, and Brazil

(9, 34). Although able to infect birds of all ages, TCoV (also referred to as bluecomb virus) causes diarrhea accompanied by anorexia and decreased weight gain in young turkeys (34). More recently, TCoV has also been associated with the poult enteritis and mortality syndrome characterized by high mortality, severe growth retardation, and immune dysfunction. TCoV is shed in feces and transmitted within and between flocks by the fecal-oral route, including mechanical transmission of feces. Infection of turkey flocks with TCoV results in decreased growth rates in poults and decreased egg production in breeder hens. Increased mortality is dependent upon age, concurrent or secondary infections, and management practices.

IBV and TCoV interspecies transmission and wild bird reservoirs

A high degree of sequence identity (85 to 90%) is present between the polymerase (ORF1b) and M and N proteins of TCoV, PhCoV, and IBV (9, 34), and IBV and TCoV are antigenically related as well (34, 47). The exception is in the S protein. IBV and PhCoV share about 90% identity in the S protein and induce similar disease syndromes in chickens and pheasants (9). In contrast, only about 34% identity exists in the S protein between IBV and the enteric TCoV strains; three TCoV strains examined shared 91% amino acid identity (63). Whether these dramatic genetic differences between the S proteins of TCoV and IBV reflect the altered enteric tropism or adaptation to a new host species for TCoV is unclear.

The close antigenic and genetic similarities between these three avian CoVs raise the possibility that they represent host range mutants as described for the group 1a CoVs (TGEV/PRCV, FCoV, and CECoV); if so, they might exhibit interspecies transmission and disease induction in heterologous hosts. However, when TCoV or PhCoV was experimentally inoculated into chickens, only asymptomatic infections were observed (32, 47). A caveat is that, as with group 1 CoVs, strain differences may influence their virulence for heterologous hosts (9, 95). In contrast, when CoVs closely related to IBV isolated from guinea fowl or teal were inoculated into chickens, disease occurred (9). Although confirming that IBV-like CoVs can replicate in nonchicken hosts, conclusions must be considered tentative because avian CoV was isolated from birds that were located in the vicinity of chickens. Thus, it is unclear if they merely represented IBV field strains acquired from nearby chickens. There is clearly a need to screen other nongallinaceous birds, including wild birds, for IBV-like or other group 3 CoVs that are infectious or emerging disease threats for domestic poultry.

PATHOGENESIS

Research on respiratory and enteric CoV infections in natural animal hosts (swine, cattle, and poultry) has provided important information on CoV disease pathogenesis, possible potentiators for increased disease severity, and vaccine strategies. In animals, CoV infections are generally most severe in the young. A notable difference between SARS-CoV and most fatal animal CoV infections is the unexplained propensity of SARS-CoV to cause more severe disease in adults than in children. However, in adult animals, respiratory CoV infections are more severe or often fatal when combined with other factors, including stress and transport of animals (shipping fever of cattle), high exposure doses, aerosols, treatment with corticosteroids, and coinfections with other respiratory pathogens (viruses, bacteria, and bacterial lipolysaccharides [LPS]). Likewise, such variables, by accentuating viral shedding or increasing the titers shed, may contribute to the phenomenon of superspreaders (a major factor in the spread of SARS) (97). The following sections provide a perspective on the pathogenesis of selected group 1, 2, and 3 enteric and respiratory CoV infections of livestock and poultry and the role of various cofactors in disease potentiation.

Group 1 CoVs

Subgroup 1a: TGEV and PRCV pathogenesis

The emergence of a naturally occurring S protein gene deletion mutant of TGEV, PRCV with an altered (respiratory) tissue tropism, has provided a unique opportunity for comparative studies of these two CoVs in the same host species (pigs). TGEV and PRCV exemplify localized enteric (TGEV) and respiratory (PRCV) infections most severe in neonatal (less than 2 weeks) and young adult (1 to 3 months) pigs, respectively (60, 95).

After exposure, presumably mainly by the fecaloral route, TGEV quickly targets small intestinal villous enterocytes, infecting virtually 100% of the small intestine, excluding the proximal duodenum (95). This rapid loss of most functional villous enterocytes leads to pronounced villous atrophy, resulting in severe malabsorptive diarrhea and potentially fatal disease in neonates (Table 1). After a short incubation period (18 to 48 h), typical clinical signs of TGE in neonates include transient vomiting and yellow, watery diarrhea. Fecal shedding of TGEV normally persists for up to 2 weeks, but in a few studies, chronic or intermittent fecal shedding by sows was detected (95). In adults, TGEV is mild, with transient diarrhea or inappetence, but curiously, pregnant or lactating animals develop more severe clinical signs, with elevated temperatures and agalactia.

Extraintestinal infections by TGEV are recognized, including infection of the mammary gland of lactating sows after natural infection or experimental injection or infusion of virus (53, 95). TGEV also infects the upper respiratory tract, with transient nasal shedding (53, 95), but infection and lesions in the lung are uncommon. Especially noteworthy, highly attenuated strains of TGEV replicate more extensively in the upper respiratory tract than in the intestine of neonatal pigs (28, 113); such attenuated strains might represent temperaturesensitive mutants selected by serial passage in cell culture. A reverse correlation was also observed between the level of attenuation of TGEV and the extent of intestinal infection. Interestingly, two nucleotide changes (at nt 214 and 655) were detected in the S protein gene between enteric (PUR46-MAD) and respiratory/enteric (PTV) TGEV strains, with the mutation in aa 219 (nt 655) most critical for altered respiratory tropism (Table 3) (3, 96). Point mutations in the S protein gene leading to a shift from an enteric to respiratory tropism were also noted in the TGEV-TOY56 strain after multiple passages in cell culture (96).

Comparisons of the entire genomic sequences of two pairs of virulent (parental) and attenuated (serial cell culture-passaged parental strain) TGEV strains

Table 3. Tissues infected by respiratory/enteric coronaviruses in animal hosts and changes in the S protein gene

Tuble 51 Tissues infected	by respiratory/enter	ie coronaviruses in animar ii	osts and enanges	in the 5 protein gen	·
T (, 1.) P.		Group 1 CoV, pigs	Group 2 CoV, cattle		
Infected tissue or condition	TGEV-V	TGEV-A (vaccine)	PRCV	BCoV-E	BCoV-R
Viremia	_	_	+	NR	NR
Upper respiratory tract	+	++	++	+	++
Lower respiratory tract	+/-	+	+++	+	+ + +
Intestine	+++	+	+/-	+++ (colon)	++ (colon)

^aTGEV-V, virulent TGEV; TGEV-A, attenuated TGEV; BCoV-E, enteric BCoV; BCoV-R, respiratory BCoV; NR, not reported; -, no infection; +/-, variable infection; +, low level of infection; ++, moderate level of infection; +++, high level of infection. In TGEV-V the S protein gene is intact. In TGEV-A, the S protein gene has point mutations at nt 214, 665, and 1753 (3, 122); in PRCV the S protein gene has a deletion of 621 to 681 nt in size; and in BCoV-E and BCoV-R the S protein gene has point mutations (16, 40), including 42 aa changes at 38 sites (40).

(Miller and Purdue) revealed a common change in nt 1753 of the S protein gene resulting in a serine-toalanine mutation at position 585 of the attenuated strains: alanine was also present in this position for the less pathogenic and respiratory PRCV-ISU-1 strain and for the attenuated TGEV-TOY56 strain (Table 3) (122). The role of this change in TGEV tissue tropism or virulence is unknown, but it resides in the major antigenic site A/B (aa 506 to 706) and the cell receptor binding domain (aa 522 to 744) of TGEV.

Like SARS-CoV, PRCV, an S protein gene deletion mutant of TGEV, spreads by droplets and has a pronounced tropism for the lungs, replicating to titers of 10^7 to 10^8 50% tissue culture infective doses (TCID₅₀) and producing interstitial pneumonia affecting from 5% to as much as 60% of the lung (21, 35, 41, 60, 95). Although many uncomplicated PRCV infections are mild or subclinical, such lung lesions are almost invariably present. Furthermore, the severity of clinical signs and the degree of pathology appear to be PRCV strain dependent. Vaughn et al. (117) suggested that PRCV strains with larger deletions in the S protein gene (PRCV-1894) had reduced pathogenicity compared to PRCV strains with smaller deletions (AR310 and LEPP).

Clinical signs of PRCV infection, like SARS, include fever with variable degrees of dyspnea, polypnea, anorexia, and lethargy and less coughing and rhinitis. Also, like for SARS-CoV (17, 70), PRCV replicates in lung epithelial cells and antigen is detected in type I and II pneumocytes and alveolar macrophages. In lungs, bronchiolar infiltration by mononuclear cells, lymphohistiocytic exudates, and epithelial cell necrosis lead to interstitial pneumonia. PRCV induces transient viremia with virus also detected from nasal swabs and in the tonsils and trachea, similar to SARS-CoV (24, 55, 76, 77). PRCV further replicates in a few undefined cells in the intestinal lamina propria, but without inducing villous atrophy or diarrhea and with limited fecal shedding. Recently, however, fecal isolates of PRCV were detected with consistent, minor (point mutations) in the S protein gene compared to nasal isolates from the same pig (20). No diarrhea and limited fecal shedding were observed in pigs inoculated with the fecal PRCV isolates, suggesting their possible lack of intestinal stability. Such observations suggest the presence of CoV quasispecies in the host (23), with some strains more adapted to the intestine, a potential corollary for the intestinal infection and fecal shedding of SARS-CoV (12, 24, 55, 61, 76, 77).

It is widely thought that the 5' deletion (621 to 681 nt in size) in the S protein gene of PRCV plays a

major role in the altered tissue tropism and reduced virulence of PRCV. Recombinant TGEV strains from infectious clones and MAb neutralization-resistant mutants have been used to try to pinpoint the molecular basis for the difference in pathogenicity and tissue tropism between TGEV and PRCV strains (3, 5, 96). Attenuated mutants of TGEV (Purdue-115 strain) selected with MAbs to S-protein site D (absent on PRCV) exhibited reduced enteropathogenicity that correlated with a point mutation or small deletion in the S protein gene encoding the N-terminal subregion (region deleted in PRCV) (5). Ballesteros et al. (3) concluded that a substitution in aa 219 of the S protein of a PUR46-MAD recombinant generated between enteric/respiratory (attenuated PUR46-MAD) and attenuated respiratory PTV (formerly NEB72) strains of TGEV resulted in the loss of enteric tropism. They speculated that this mutation affected virus binding to an intestinal coreceptor.

Use of infectious clones of TGEV and PRCV may further assist in identifying other genes that influence CoV tropism and virulence. Amino acid changes in the M protein affect alpha interferon (IFN- α) induction by the attenuated Purdue-P115 strain, implying a potential role in altered host response and virulence (59). The deletions or mutations observed in nonstructural proteins 3a and 3b of all PRCV strains may lead to nonexpression or may render these proteins nonfunctional (75). An infectious clone of PUR46-MAD with ORF3 gene deletions showed a slightly reduced pathogenicity in vivo (but the initial strain used was already attenuated) but normal replication in cell culture (100). Using IBV infectious clones, similar effects of ORF3 deletion were reported (45). Although a TGEV strain (96-1933) with an ORF3a deletion maintained pig virulence, the virus isolated and sequenced was not plaque purified and tested in pigs to confirm the presence of a single strain of TGEV (67). This is important because of previously reported mixtures of virulent and attenuated TGEV strains in an early in vivo passage of the virulent Purdue strain of TGEV (96).

Subgroup 1b: PEDV pathogenesis

The pathogenesis (including clinical signs, lesions and disease) of PEDV in the small intestine of piglets closely resembles that of TGEV (80), but with a lower rate of replication and longer incubation period. However, unlike with TGEV, colonic villi were also infected, with cellular changes in colonic enterocytes containing PEDV particles in older swine. Features of PEDV infection not seen in TGE are acute back muscle necrosis and, occasionally, sudden death in finishing and adult pigs.

Group 2 CoVs

Subgroup 2a: BCoV pathogenesis

Besides severe pneumonia (70), shedding of SARS-CoV in feces and the occurrence of diarrhea in many patients (12, 61, 76) suggest that SARS-CoV may be pneumoenteric like BCoV. Enteric and/or respiratory shedding of virus was also detected in wild animals harboring SARS-CoV (Table 1) (33, 58, 62). Thus, a review of the pathogenesis of BCoV in cattle in the context of each of the three clinical syndromes (Table 2) provides comparative data on another group 2 CoV with dual respiratory and enteric tropism.

Calf diarrhea BCoV strains infect the epithelial cells of the distal small and large intestine and superficial and crypt enterocytes of the colon, leading to villous atrophy and crypt hyperplasia (Table 2) (92, 114). As summarized in Table 2, after an incubation period of 3 to 4 days, calves develop a severe, malabsorptive diarrhea persisting for 2 to 8 days, resulting in dehydration and often death. Concurrent fecal and nasal shedding often occur, and most diarrheic calves necropsied have BCoV antigen in both intestinal and respiratory (turbinate, nasal, and tracheal) epithelial cells. These results were also confirmed by experimental BCoV challenge studies of calves (84, 94). Saif et al. (94) reported intestinal lesions in all calves inoculated with an enteric BCoV calf isolate (DB2), but lesions (focal emphysema) and BCoV antigen in the lung were less frequent (20 to 30% of calves). Thus, enteric strains of BCoV induce diarrhea and are potentially pneumoenteric, but respiratory disease is variable.

BCoV is also implicated as a cause of mild respiratory disease (coughing and rhinitis) or pneumonia in 2- to 6-month-old calves, and viral antigen is detected in nasal secretions, the lungs, and often the intestines (68, 84). Experimental calf challenge studies using calf respiratory BCoV isolates confirmed both fecal and nasal shedding and diarrhea but only variable respiratory disease (68, 84). However, in the field, as described below, respiratory BCoV infections are likely exacerbated by stress or respiratory coinfections.

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 (42, 94, 106). 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. A similar scenario may prevail for the SARS-CoV to explain its pneumoenteropathogenicity with prominent fecal shedding and diarrhea in some patients (12, 61, 76).

For WD, intestinal lesions and BCoV-infected cells in the colonic crypts of dairy and beef (feedlot) cattle resemble those described for calf diarrhea (Table 2) (13, 114). The disease is acute, with only transient BCoV shedding detected in feces (1 to 4 days) by immuno-EM or ELISA (13, 86). BCoV isolates from WD outbreaks at least partially reproduced the disease (diarrhea) in BCoV-seropositive nonlactating dairy cows (110) and more authentically (bloody diarrhea and decreased milk production) in BCoV-seronegative lactating dairy cows (107). Interestingly, in the latter study, older cattle were more severely affected than similarly exposed calves, mimicking the more severe SARS cases seen in adults versus children (76).

In these two experimental studies, the incubation period ranged from 3 to 8 days and diarrhea persisted for 1 to 6 days. 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 (110). In contrast, BCoV-seronegative cows directly exposed to calves experimentally infected with a WD strain of BCoV developed transient fevers, mild cough, and serous mucopurulent discharge (107). These data are consistent with field outbreak reports indicating variable signs of respiratory disease in cattle with WD (13, 86). They further document the very short shedding window (1 to 4 days) for detection of BCoV in feces, showing the need for acute-phase samples for definitive BCoV diagnosis or the testing of seroresponses to BCoV on a herd basis (86). As reviewed in the BCoV epidemiology section, WD isolates of BCoV are antigenically closely related to calf enteric and respiratory isolates, and the various strains, regardless of clinical origin, elicit cross-protection against one another in calf challenge studies (4, 14, 26, 38).

The BRDC of feedlot calves is most pronounced during the first weeks after arrival at feedlots. This overlaps with a high prevalence of respiratory viral and secondary bacterial coinfections and various environmental or host stress factors during this period (see below). During the past decade a growing number of reports have provided epidemiological or experimental evidence suggesting that BCoV infection contributes substantially to the BRDC. Multiple previous studies (reviewed earlier) have documented both nasal and fecal shedding of BCoV by calves shortly after arrival in feedlots and subsequent seroconversion to BCoV (Table 2). Storz et al. (102) showed a progression in development of the BRDC for natural cases, 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 pneumonia and deaths in 26 cases, most of which involved concurrent high titers of BCoV and bacteria in the lungs. The authors concluded that these data supported a role for BCoV in the BRDC as defined by Evans' criteria for causation. In this and another study (103), researchers confirmed the presence of BCoV antigen in respiratory epithelial cells or isolated BCoV from nasal secretions, trachea, bronchi, or lung aveoli and documented the presence of interstitial emphysema, bronchiolitis, and aveolitis in concert with bacterial infection (Table 2). Thus, the BRDC appears to be a multifactorial disease, with BCoV playing an early role in inciting the disease. Although multifactorial/multiagent experiments describing interactions among respiratory viruses, bacteria, and stress have highlighted their synergistic effects in the BRDC (51), no such experiments have been reported for BCoV. Such studies of BCoV-seronegative, stressed calves of feedlot age are needed to further elucidate the role of BCoV in the BRDC and the mechanisms involved in predisposing calves to development of fatal pneumonia.

Unlike the scenario for the almost exclusive respiratory tropism of PRCV, no deletions have been detected in the S protein gene of respiratory BCoV strains, most of which also possess an enteric tropism as revealed by calf challenge studies (14). Several groups have compared the S (or S1) protein gene sequences of WD or respiratory and enteric BCoV isolates, including isolates from the same animal (16, 29, 40, 52, 121). Focusing on the hypervariable region (aa 452 to 593) containing the neutralizing epitope (S1B) of the S1 subunit, four groups (16, 29, 40, 121) reported that respiratory strains (or respiratory and enteric isolates from the same feedlot calf) had changes in aa 510 and 531 compared to the reference enteric Mebus 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 two studies (16, 121), but not in another (40). Others (52) reported that the S protein gene sequences of Korean WD BCoV isolates were similar to those of respiratory and enteric BCoV strains. Nevertheless, all investigators showed the greatest amino acid sequence divergence (42 aa changes at 38 distinct sites [40]) and most differences by phylogenetic analysis between the historic (1972) reference Mebus enteric BCoV and more recent BCoV isolates, regardless of clinical

origin. The relevance of the observed genetic changes to viral pathogenesis in vivo is unknown. Although Hasoksuz et al. (38) observed antigenic and biological differences among BCoV isolates, variability was not necessarily related to the clinical origin of the isolates. Because BCoV, like other RNA viruses, represents a quasispecies (23), some viruses within the quasispecies may be more suitable for respiratory than intestinal replication, contributing to sequence changes reported for such isolates from the same host (20).

Group 3 CoVs

IBV pathogenesis

IBV replicates at multiple epithelial surfaces of chickens, including ones not associated with pathology (alimentary tract and gonads) and those associated with pathology (respiratory tract [nose, trachea, lungs, and air sacs], kidney [tubes and ducts], and oviduct) (8, 10, 19). IBV replicates mainly in epithelial cells, causing necrosis and edema with small areas of pneumonia near large bronchi in the respiratory tract and interstitial nephritis in the kidney (8, 10, 19). In these infected tissues, there is a loss of cilia from epithelial cells, edema, and various degrees of mononuclear cell or heterophil infiltration. IBV strains differ in virulence, and only a small percentage of IBV strains are highly nephropathogenic. Of interest for SARS is the persistence of IBV in the kidney and its prolonged fecal shedding, because SARS-CoV is also detected in urine and shed longer term in feces (76, 77). However, it is unclear if SARS-CoV shedding in urine is a consequence of viremia or a kidney infection like with IBV. The genetic basis for these differences in IBV strains is unclear, but serial passage of a respiratory IBV strain via the cloacal route resulted in an altered kidney tropism (111). The extent of mortality from IBV infection is age dependent (greatest in chicks) and influenced by the chickens' genetic background (2).

IBV is recovered intermittently from the respiratory tract for about 28 days after infection and from the feces after clinical recovery (20 weeks postinoculation) (8, 10, 19). The cecal tonsil and the kidney are possible reservoirs for IBV persistence, similar to the persistence of FCoV in the intestine of cats (44). IBV was recovered from both tracheal and cloacal swabs from chickens at onset of egg production, suggesting reexcretion of IBV from chronically infected, stressed (coming into lay) birds, as also demonstrated for fecal shedding of FCoV or BCoV after induction of immunosuppression (71, 110). The ability of IBV strains to persist in the host provides a longer opportunity for new mutants to be selected with altered tissue tropisms and virulence from among the viral RNA quasispecies (23). The persistence of IBV and its replication in multiple tissues may contribute to the sequence diversity in the IBV S protein and explain the vast array of IBV serotypes, a notable difference compared to only one or two serotypes for most group 1 or 2 CoVs.

TCoV pathogenesis

TCoV infection is associated mainly with diarrhea and weight loss in young poults or a drop in egg production in breeder hens (34, 49). Replication of TCoV is confined to the apical enterocytes of the intestinal villi and the epithelium of the bursa of Fabricius. Like other enteric CoVs, TCoV causes a malabsorptive, maldigestive diarrhea presumably due to viral destruction or functional alterations in infected enterocytes. Severe disease and high mortality were features of early TCoV infections (bluecomb) and experimental studies using crude fecal or intestinal homogenates; recent studies using embryo-propagated TCoV strains indicate negligible mortality and moderate growth depression (49). Similar to the role of BCoV in the BRDC, interactions between TCoV and other agents (E. coli, astrovirus, etc.) in the development of more severe enteric disease have been described (34, 49). TCoV is shed in feces of turkeys for several weeks after recovery from clinical disease and was detected in intestinal contents for 6 and 7 weeks postinoculation by virus isolation and RT-PCR, respectively.

COFACTORS THAT EXACERBATE CoV 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 group 1 (TGEV or PRCV), group 2 (BCoV), and group 3 (IBV or TCoV) CoV infections. In addition, similar cofactors may enhance the severity of SARS-CoV or play a role in the superspreader cases seen in the SARS epidemic (97) by enhancing virus transmission or host susceptibility.

Impact of Respiratory Coinfections on CoV Infections, Disease, and Shedding

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 (15, 37, 56, 57, 102–104, 106). The BRDC can be precipitated by several viruses, alone or in combination (BCoV, bovine respiratory syncytial

virus, parainfluenza 3 virus, and bovine herpesvirus), including viruses similar to common human respiratory viruses and viruses capable of mediating immunosuppression (bovine viral diarrhea virus, etc.). The shipping of cattle long distances to feedlots and the comingling of cattle from multiple farms create 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. Such factors are analogous to the physical stress of long airplane trips with close contact among individuals from diverse regions of the world, both of which may play a role in enhancing an individual's susceptibility to SARS or the transmission of SARS-CoV (73, 120). For the BRDC, various predisposing factors (viruses and stress) allow commensal bacteria of the nasal cavity (Mannheimia haemolytica, Pasteurella, Mycoplasma, etc.) to infect the lungs, leading to a fatal fibrinous pneumonia (56, 102–104) like that seen in SARS patients (17, 70, 77).

In broiler chickens, severe disease or death ensues from systemic *E. coli* coinfections after IBV damage to the respiratory tract or *Mycoplasma* coinfections with IBV (10, 19). Similarly for the enteric TCoV, coinfection with either enteropathogenic *E. coli* or astrovirus led to enhanced disease severity compared to that caused by each agent alone (34, 49).

It is also possible that antibiotic treatment of such animals (or SARS patients) coinfected with CoVs and bacteria, with massive release of bacterial LPS, could precipitate induction of proinflammatory cytokines, which could further enhance lung damage. For example, Van Reeth et al. (115) showed that pigs infected with PRCV followed by a subclinical dose of E. coli LPS within 24 h developed enhanced fever and more severe respiratory disease compared to those obtained with each agent alone. They concluded that the effects were likely mediated by the significantly enhanced levels of proinflammatory cytokines induced by the bacterial LPS. Thus, there is a need to examine both LPS and lung cytokine levels in SARS patients as possible mediators of the severity of SARS. Bacteria (Chlamydia spp.) have been isolated from SARS patients, but their role in enhancing the severity of SARS is undefined (83).

Interactions between PRCV and other respiratory viruses may also parallel the potential for concurrent or preexisting respiratory viral infections to interact with SARS-CoV (such as metapneumoviruses, influenza viruses, reoviruses, respiratory syncytial virus, or OC43 or 229E CoV). Hayes (41) showed that sequential dual infections of pigs with the arterivirus porcine respiratory and reproductive syndrome virus, followed in 10 days by PRCV, significantly enhanced lung lesions and reduced weight gains compared to those obtained with each virus alone. The dual infections also led to more pigs shedding PRCV nasally for a prolonged period and, surprisingly, to fecal shedding of PRCV. The lung lesions observed resembled those in SARS victims (17, 70).

In another study, Van Reeth and Pensaert (116) inoculated pigs with PRCV followed in 2 to 3 days by swine influenza A virus (SIV). They found that SIV lung titers were reduced in the dually infected compared to the singly infected pigs, but paradoxically the lung lesions were more severe in the dually infected pigs. They postulated that the high levels of IFN- α induced by PRCV may mediate interference with SIV replication, but might also contribute to the enhanced lung lesions. Such studies are highly relevant to possible dual infections by SARS-CoV and influenza virus and the potential treatment of SARS patients with IFN- α .

Impact of Route (Aerosols) and Dose on CoV Infections

Experimental inoculation of pigs showed that administration of PRCV by aerosol compared to the oronasal route, or in higher doses, resulted in shedding of higher virus titers for longer times (112, 113). In other studies, high PRCV doses induced more severe respiratory disease. Pigs given $10^{8.5}$ TCID₅₀ of PRCV had more severe pneumonia and a higher mortality rate than pigs exposed by contact (50), and higher intranasal doses of another PRCV strain (AR310) induced moderate respiratory disease, whereas lower doses produced subclinical infections (35). By analogy, hospital procedures that could potentially generate aerosols, or exposure to higher initial doses of SARS-CoV, may enhance SARS transmission or lead to enhanced respiratory disease (101).

Impact of Treatment with Corticosteroids on CoV Infections of Animals

Corticosteroids are known to induce immunosuppression and reduce the numbers of CD4 and CD8 T cells and certain cytokine levels (30). Many hospitalized SARS patients were treated with steroids to reduce lung inflammation, but there are no data to assess the outcome of this treatment on virus shedding or respiratory disease. A recrudescence of BCoV fecal shedding was observed in one of four WD BCoVinfected cows treated with dexamethasone (110). Similarly, treatment of older pigs with dexamethasone prior to TGEV challenge led to profuse diarrhea and reduced lymphoproliferative responses in the treated pigs (98). These data raise issues related to corticosteroid treatment of SARS patients and possible secondary immunosuppression and increased and prolonged CoV shedding. Alternatively, corticosteroid treatment may be beneficial in reducing proinflammatory cytokines if found to play a major role in lung immunopathology (30).

IMMUNITY AND VACCINES

As reviewed in the preceding section, an understanding of the pathogenesis of CoV infections, including the target organs infected and how virus is disseminated to these organs, has assisted in development of vaccine strategies. The realization that these animal CoVs cause the most severe disease in young animals and primarily infect epithelial cells lining the respiratory and/or intestinal tracts has necessitated development of mucosal vaccines effective at the local site of infection in neonates or young birds (TGEV, BCoV, and IBV) as well as in juveniles or adults (BCoV).

Development of safe and efficacious and new bioengineered vaccines for animal CoV infections has been problematic and only partially successful (see reviews in references 87 to 89 and 93). Problems encountered often relate to a lack of understanding of basic mechanisms of induction of mucosal immunity. Stimulation of protective mucosal immunity, especially priming of seronegative vaccinees, often requires use of live replicating vaccines or vectors as opposed to nonreplicating killed viruses or subunit vaccines (unless applied with effective mucosal delivery systems or adjuvants), to provide optimal mucosal antigenic stimulation and to avoid tolerance induction (87-89). In addition, most vaccines for mucosal pathogens may fail to induce sterilizing immunity or to prevent respiratory reinfections, as commonly observed for natural respiratory CoV infections (PRCV, IBV, BCoV, and human CoV); consequently, the major vaccine focus is often to prevent severe disease.

Enteric CoV Vaccines

Because CoV-induced diarrheal disease is most severe in young animals, the major target for TGEV and BCoV vaccines is the suckling animal (reviewed in references 87 to 89, 93, and 95). These CoV vaccines are designed to immunize dams, with passive transfer of antibodies in milk to nursing offspring to prevent intestinal infections.

Passive immunity to TGEV

Key new concepts related to mucosal immunity have originated from previous studies of passive immunity to TGEV infections in pigs (see reviews in references 87 to 89 and 95). Early studies revealed that sows that recovered from natural TGEV infections provided protective lactogenic immunity to their suckling pigs via neutralizing secretory IgA (sIgA) antibodies in milk. These protective sIgA antibodies were stimulated in milk only after an intestinal TGEV infection of the mother (sow), leading to the initial concept of a gut-mammary-sIgA immunologic axis (specific trafficking of IgA immunoblasts from the gut to the mammary gland) and an interconnected common mucosal immune system linking distant mucosal tissues.

In monogastric animals (pigs and humans) which secrete sIgA antibodies in milk, passive immunization against enteric pathogens is accomplished by exploiting the common mucosal immune system. Because neutralizing TGEV sIgA antibodies in milk are a correlate of protection in neonatal pigs, the strategy is to evoke the gut-mammary IgA axis by administering attenuated TGEV (TGEV-A) vaccines orally to induce sIgA antibodies in milk via intestinal stimulation of the mother. Problems encountered in the field application of this strategy were as follows (see reviews in references 87 to 89 and 95). First, the commercial TGEV-A vaccines given intranasally or orally were of low titer ($>10^6$ PFU) and replicated poorly in the adult intestine, so few IgA precursor B cells were induced in the intestine (112, 113) for subsequent migration to the mammary glands, leading to low IgA antibody titers in milk and only partial passive protection. Use of less attenuated TGEV strains or the antigenically related FIPV caused disease in baby pigs. These studies further illustrate the difficulty in priming for protective sIgA mucosal immune responses, even using live vaccines in naïve seronegative adult animals. However, in comparison, killed TGEV vaccines given parenterally (intramuscularly) induced only IgG antibodies in milk and no passive protection of suckling pigs against TGEV.

The emergence of PRCV also permitted comparative immunologic studies of immune responses and protection against enteric TGEV versus respiratory PRCV in the porcine host. Both maternal vaccination to provide passive antibodies in milk to suckling pigs and active immunity have been evaluated (6, 89, 95, 112, 113). The finding from the PRCV/TGEV pig studies that stimulation at one mucosal site (respiratory tract) does not necessarily evoke complete reciprocity in immune responses or protection at a distant mucosal site (intestine) led to a second important concept: compartmentalization exists within the common mucosal immune system. A single infection of the respiratory tract of pigs or sows with PRCV induced only partial active or passive immunity to TGEV, respectively (95, 112). This finding and results of other studies (reviewed in reference 95) suggest that repeated PRCV exposure is needed to stimulate adequate numbers of IgA memory cells in the intestine, for local gut protection, and for their subsequent transit from intestine to the mammary gland, with secretion of protective levels of IgA antibodies in milk.

For young pigs, VanCott et al. (112, 113) found that a single PRCV infection of the respiratory tract induced a few IgA antibody-secreting cells in the intestine but higher numbers of IgG antibody-secreting cells in the lower respiratory tract (bronchial lymph nodes). Importantly, PRCV infection primed for anamnestic IgG and IgA intestinal antibody responses after TGEV challenge, leading to partial protection. In the field, pigs experience multiple respiratory infections with PRCV, providing sufficient immunity to TGEV such that TGE has largely disappeared from European swine herds (60, 95). The relevance of these vaccine studies to SARS is that if SARS-CoV frequently causes primary intestinal infections, besides pneumonia, neither killed parenteral nor respiratory vaccines may prevent diarrheal disease or fecal shedding, or respiratory vaccines may require high and repeated doses to elicit intestinal immunity.

Use of parenteral TGEV subunit vaccines (baculovirus-expressed S glycoproteins) to induce active immunity to TGEV-induced diarrhea in weaned pigs elicited neutralizing antibodies in serum but failed to induce protection against TGEV-induced diarrhea (87-89, 95). The data confirm earlier findings using killed TGEV vaccines parenterally that indicated that serum neutralizing antibodies (in contrast to intestinal or milk sIgA antibodies) do not correlate with a high degree of protection against TGEV infection. However, in a subsequent study, partial protection against TGEV infection (fecal shedding) was induced in pigs vaccinated intraperitoneally with the S glycoprotein mixed with the N and M proteins. Other studies of TGEV also suggested that both recombinant N proteins (T-cell epitopes) and S proteins were required for maximal antibody responses to TGEV (1). Thus, in spite of long-term research efforts, effective TGEV enteric vaccines have remained elusive, but with the emergence of PRCV, nature appears to have generated its own highly effective vaccine to moderate the more virulent TGEV infections.

Respiratory CoV Vaccines

In spite of its economic impact, no respiratory CoV vaccines have been developed to prevent BCoVassociated pneumonia in calves or in cattle with the BRDC. The correlates of immunity to respiratory BCoV infections remain undefined. Limited data from epidemiological studies of BCoV infections in cattle suggest that serum antibody titers to BCoV may be a marker for respiratory protection. Antibody isotype (IgG1, IgG2, and IgA), neutralizing antibody titer, and magnitude of antibody titer in serum of naturally infected calves or in cattle on arrival in feedlots were correlated in multiple studies with protection against respiratory disease, pneumonia, or BCoV respiratory shedding (15, 43, 64, 66, 106). However, whether the serum antibodies are themselves correlates of respiratory protection or only reflect prior enteric or respiratory exposure to BCoV is uncertain. In a recent study, intranasal vaccination of feedlot calves with a modified live BCoV calf vaccine on entry to a feedlot reduced the risk of the BRDC in calves (81).

The only available animal CoV vaccines targeted to prevent respiratory CoV infections (bronchi) are IBV vaccines for chickens. Both live attenuated and killed commercial IBV vaccines are used (8, 10, 19). Attenuated vaccines are used in broilers, usually at 1 day of age and 10 days later, because only short-term (6 to 7 weeks) protection is needed. Attenuated vaccines are administered by evedrop or intranasally or, for mass application, by aerosol or in drinking water. For layers or breeders for which longer protection is needed (\sim 18 months), attenuated vaccines are used for priming at 2 to 3 weeks of age followed by injection of killed oil emulsion booster vaccines, often at 8- to 10-week intervals throughout the laying cycle. The correlates and mechanisms of protection against IBV clinical disease are uncertain. High levels of neutralizing antibodies are thought to prevent viral dissemination from the respiratory tract, thus blocking secondary virus infection of the reproductive tract and kidneys (19). Also, high levels of maternal antibodies (yolk derived) prevented IBV infection of chicks for the first 2 weeks of life. These data suggest that neutralizing antibodies play a role in mediating protection against IBV. However cell-mediated immunity also plays a role in protection to IBV, as shown by the ability of adoptively transferred α/β CD8 T cells to protect chicks against IBV challenge (reviewed in references 8 and 10). Evidence suggests that the S1 glycoprotein and N protein (expressed in vectored or DNA plasmid vaccines) of IBV can induce variable levels of protection. The S1, N, and M proteins also induced cell-mediated immune responses to IBV. Problems encountered in vaccine protection against IBV include the existence of multiple serotypes or subtypes of IBV which may fail to cross-protect, variation in virulence among IBV field strains, and the possible increase in virulence of some live vaccines after backpassage in chickens, with the suggestion that point mutations in the genomes of attenuated vaccines may generate new epidemic strains of IBV (8, 10).

SUMMARY AND CONCLUSIONS

In summary, as highlighted in this chapter, much progress has been made in the topics addressed, encompassing the comparative biology of animal CoVs. As documented, enteric coronaviruses alone can cause fatal infections in seronegative young animals. However, in adults respiratory CoV infections are more often fatal or more severe when combined with other factors, including high exposure doses, aerosols, treatment with corticosteroids, and respiratory coinfections (viruses, bacteria, and LPS). These variables may also influence the severity of disease or contribute to the phenomenon of superspreaders. Long-term and recent studies of animal CoVs have highlighted the potential for new CoV strains to emerge as deletion mutants or recombinants from existing strains or for new strains to appear from unknown or perhaps wildlife reservoirs; the latter is a likely origin for SARS-CoV. A number of CoV strains, particularly ones from wild animals, remain to be characterized, and the full genomic sequence is available for only a relatively small number of human and animal CoVs outside of SARS-CoVs. In addition, interspecies transmission of certain CoVs may not be uncommon, although the determinants of host range specificity among CoVs are undefined.

An understanding of CoV disease pathogenesis is critical for the design of effective vaccine strategies, yet many unanswered questions related to CoV disease mechanisms remain, including factors that exacerbate CoV respiratory disease or shedding. Consequently, development of safe and efficacious vaccines for animal CoV infections has been problematic and only partially successful. Problems encountered also often relate to a lack of understanding of basic mechanisms to induce mucosal immunity by vaccines targeted at preventing enteric or respiratory mucosal infections. Stimulation of protective mucosal immunity, especially priming of seronegative vaccinees, often requires use of live replicating vaccines or vectors as opposed to nonreplicating killed viruses or subunit vaccines (unless applied with effective mucosal delivery systems or adjuvants). These approaches mimic natural CoV infections and provide optimal mucosal antigenic stimulation while avoiding tolerance induction (87-89, 95). In addition, vaccines for mucosal pathogens may fail to induce sterilizing immunity or prevent reinfections, as commonly observed for natural CoV respiratory infections; therefore, the major vaccine focus may be to prevent severe disease. For both TGEV and IBV infections, live vaccines alone (TGEV) or for priming, followed by killed vaccines for boosting (IBV), provided at least partial protection against enteric and respiratory

disease, respectively (8, 89, 93, 95). But as illustrated for IBV, live vaccines may revert to virulent if inadequately attenuated, raising safety issues.

We are embarking on a new era in CoV research attributable to both new discoveries and technological advances. First is the recent discovery and characterization of new CoV strains, including SARS-CoVs from humans and wildlife species. In parallel, the increasing availability of full length CoV genomic sequence data (30 kb), including data for virulent and attenuated CoV pairs or for original and adoptive host strains, presents new opportunities for comprehensive genomic and phylogenetic analyses. Finally, the recent development and application of reversegenetics systems for CoVs should yield further breakthroughs in understanding the genetic basis for CoV pathogenicity and targets for the rational design of attenuated CoV vaccines.

What is lagging for further advancements in CoV research are appropriate animal models to study respiratory and/or enteric human CoV infections, including SARS-CoV, and a more comprehensive and in-depth understanding of the comparative pathogenesis and disease mechanisms related to CoV infections in natural or adoptive hosts. For example, the basic mechanisms of how CoVs induce a broad spectrum of respiratory disease (ranging from mild to fatal), age-related factors in disease susceptibility, and cofactors that exacerbate disease or lead to enhanced shedding and transmission (superspreaders) are unclear, and these difficult in vivo animal studies have been undertaken by only a few investigators. Also unknown is why some CoVs cause severe disease in neonates or in adoptive hosts but mild disease in adults or natural hosts, or vice versa.

Relatively little is known about the basis for interspecies transmission of CoVs or why some CoVs (group 2 BCoVs and SARS-CoVs) have broad host ranges. Although CoVs have been and continue to be recovered from wildlife reservoirs, with some strains closely related to human (SARS-CoV) and animal (BCoV) CoVs, we understand little about their ecology in the wildlife reservoir (including apparently health animals) or their potential to emerge as either public or animal health threats.

Finally, although we have successfully bioengineered CoV subunit and live vectored vaccines, with CoV vaccines based on infectious clones on the horizon, a basic lack of understanding of the induction of immunity at mucosal surfaces, the attributes of effective mucosal vaccines, and the immune correlates of protection for CoV infections remains a major obstacle to development of effective CoV and other mucosal vaccines.

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