Chapter 12

Comparative Biology of Animal Coronaviruses: Lessons for SARS

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Introduction

The genus *Coronavirus* is comprised of at least three antigenic and genetic groups of coronaviruses (CoV) that cause mild to severe enteric, respiratory or systemic disease in domestic animals, wild animals, poultry and rodents, and minor colds in humans (Table 12.1). The newly emerged SARS CoV is probably one distantly related to group 2 viruses (Chapter 8). A morphological distinction between some group 2 CoVs and the other CoV groups is a double layer of surface projections, the shorter haemagglutinin (HE) and the longer spike (S) as apparent for bovine CoV (Fig. 12.1A) compared with only the spike for TGEV (a group 1 virus) (Fig. 12.1B; Table 12.1). SARS CoV lacks the HE surface protein. It has been reported[1] that polyclonal antisera to the group 1 CoVs, transmissible gastroenteritis virus (TGEV) and feline infectious peritonitis virus (FIPV), but not to group 2 CoVs, cross-reacted with SARS CoV-infected cells, suggesting a potential antigenic relationship with group 1 CoV. Preliminary data (XJ Meng, personal communication) suggest that this cross-reactivity may reside in the N protein. A precursor animal SARS-like coronavirus has been identified (Chapter 11) suggesting that SARS coronavirus emerged from an animal reservoir. Other new human and animal group 1 coronaviruses have been recently documented.[2–4]

The emergence of SARS illustrates that CoV can cause potentially fatal disease in humans as previously recognized for animal CoVs (Table 12.1). Because pneumonia and diarrhoea occur in SARS patients, this review will focus on animal CoVs that cause respiratory or respiratory/enteric disease (Table 12.1) because these may provide an insight into the pathogenesis and evolution of SARS.

Coronavirus evolution and pathogenesis

Group 1 porcine CoVs: models for enteric or respiratory CoV disease

New strains with altered tissue tropism can arise from existing strains through mutation. For example, the porcine respiratory coronavirus (PRCV) is a less virulent variant of TGEV and feline infectious peritonitis virus (FIPV) is the virulent variant of feline enteric coronavirus (FECoV).[5,6] Alternatively, new strains may occur after recombination events such as the potential S gene recombinants between canine coronavirus (CCoV) and FECoV type 1 leading to a new FECoV serotype (type 2)[6,7] or the acquisition of an influenza group C-like haemagglutinin (HE) by BCoV or its CoV ancestor.[8] In addition, like SARS, new animal strains have emerged from unknown sources such as the porcine epidemic diarrhoea virus (PEDV) that first appeared in Europe and Asia between 1978 and the 1980s. It initially caused high diarrhoeal mortality in suck-

Table 12. 1 Coronavirus genetic and antigenic groups, target tissues and diseases

Genetic group	Virus	Host	Within-group antigenic cross-reactions[a]				Disease/infection site		
			VN	CoV	N	S	Respiratory	Enteric[b]	Other[c]
I	HCoV-229E	Human	–	+	+	?	X upper		
	TGEV	Pig	+	+	+	+	X upper	X SI	
	PRCV	Pig	+	+	+	+/–	X upper/lower		Viraemia
	PEDV	Pig	–	?	+	?		X SI, Colon	
	FIPV	Cat	+	+	+	+	X upper	X SI	Systemic
	FCoV	Cat	+	+	+	+		X SI	
	CCoV	Dog	+	+	+	+		X SI	Systemic
	RaCoV	Rabbit	–	+	?	?			
	HCoV-NL63	Human	?	?	?	?			
II	HCoV-OC43	Human	?	+	+	+/–	X upper	?? (BCoV?)	
	MHV	Mouse	?	?	?	–		X	Hepatitis, CNS, systemic
	RCoV (sialodocry-adenitis)	Rat	?	?	?	–	X		Eye, salivary glands
	HEV	Pig	?	+	?	?	X		CNS
	BCoV**	Cattle	?	+	+	+/–	X lung	X SI, Colon	
III	IBV	Chicken	?	+	?	?	X upper	X	Kidney Oviduct
	TCoV (TECoV)	Turkey	?	+	?	?		X SI	
Pending classification	SARS	Human	+	+	+	?	X lung	X SI, colon	Viraemia, kidney?
	Civet cat CoV	Himalayan palm civet	+	+	+	?	X	X	Subclinical?
Putative group 2	Raccoon dog CoV	Raccoon dog	+	+	+	?	?	X	Subclinical?

[a]One or two-way cross-reactions by virus neutralization (VN), and in serologic tests (ELISA, IF) versus intact virus (CoV), the nucleoprotein (N) or the spike (S) protein including cross-reactivities defined using monoclonal antibodies.? = unknown or unreported.

[b]SI = small intestine; ?? = BCoV-like CoV from a child, Zhang *et al.* 1994.[40]

[c]CNS = central nervous system.

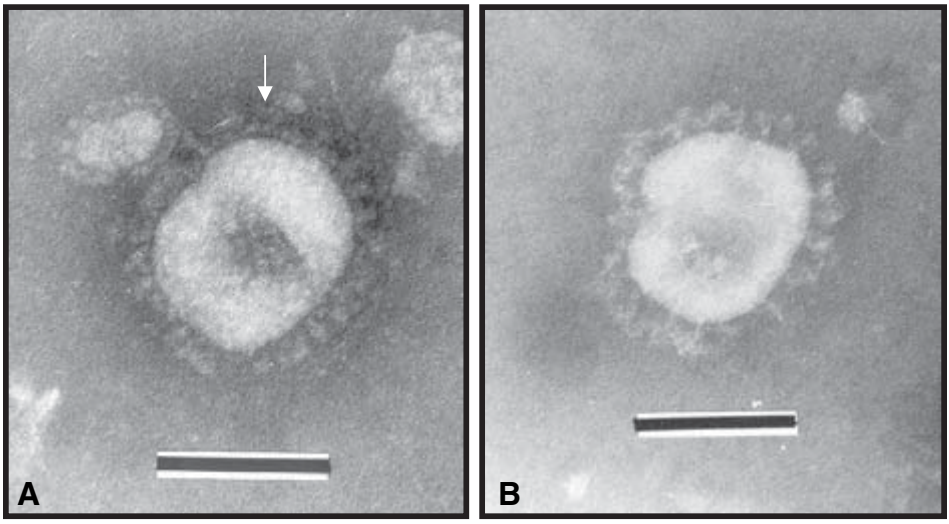

Figure 12.1 Electron micrograph of negatively stained CoV particles. (A) A typical BCoV particle showing shorter HE (top of particle, arrow) and longer S (bottom and sides) surface projections. (B) A typical TGEV particle showing single layer of longer S surface projections, similar to SARS CoV. Bar represents 100 nm.

ling pigs, but it is now endemic causing only mild or subclinical infections in previously PEDV seropositive herds.[9] PEDV appears to be more closely related to human CoV 229E than to the other animal group 1 CoVs[10] and, like SARS, but unlike the other group 1 CoVs, it grows in Vero cells.[11]

TGEV and PRCV infections as models for changes in tissue tropism

TGEV (Fig. 12.1B) causes potentially fatal gastroenteritis in young pigs, targeting the small intestinal epithelial cells, and leading to severe villous atrophy and malabsorptive diarrhoea (Tables 12.1 and 12.2). The virus also replicates in the upper respiratory tract with transient nasal shedding,[12] but infection or lesions in the lung are uncommon.[13] The disease is mild in adults with transient diarrhoea or inappetence, but pregnant or lactating animals develop more severe clinical signs including agalactia[13] similar to winter dysentery CoV infections in dairy cattle.[14,15]

Deletion mutants of TGEV with varying size S gene deletions (PRCV) appeared independently in Europe in 1984 and in the US in 1989[13,15] with a pronounced tropism for the lower respiratory tract and little intestinal replication. A major deletion occurred at the 5′ end of the S gene (nt 45–752), ranging from 621–681 nt in size. Smaller deletions occurred preceding or in ORF 3a (encoding an undefined NS protein) leading to its lack of expression.[13] Otherwise TGEV and PRCV viruses share high nt (96%) sequence identity. This is reminiscent of SARS CoV and its precursor animal virus where the human strains have acquired a deletion in the ORF8 gene region.[16] Truncation of the S gene of TGEV also led to loss of antigenic site D, permitting use of monoclonal antibodies (MAbs) to site D to differentiate serologically TGEV and PRCV antibodies by blocking ELISA.[13] Conventional virus neutralization (VN) tests do not discriminate between these viruses because the immunodominant neutralizing antigenic site (A) is conserved on PRCV and TGEV.

The altered tissue tropism (respiratory)

Table 12.2 Tissues infected by respiratory/enteric coronaviruses in animal hosts or models and changes in the S gene

Infected tissues	**Coronavirus**							
	Macaque[a]	**Cat**[a]	**Ferret**[a]	**Pigs**	**TGEV-A (vaccine)**	**PRCV**	**Cattle**	
	SARS	**SARS**	**SARS**	**TGEV-V**			**BCoV-E**	**BCoV-R**
Viraemia	NR[b]	NR	NR	–	–	+	NR	NR
Upper respiratory tract	+	+	+	+	++	++	+	++
Lower respiratory tract	+	+	+	+/–	+	+++	+	+++
Intestine	+/– (1/4)	+/–	+/–	+++	+	+/–	+++ (colon)	++ (colon)
S- gene	Intact	Intact	Intact	Intact	Pt mutations[c] (nt 214 and 655)	Deletion (621–682 nt)	Pt mutations[d,e] (42 aa changes at 38 sites)[e]	

[a]Chapters 7 and 10; [b]NR = Not reported; [c]Ballesteros *et al.*, 1997;[17] [d]Hasoksuz *et al.*, 2002;[36] [d,e]Chouljeuko *et al.*, 2001.[36a]

and reduced virulence of the PRCV variant have been attributed mainly to the S gene deletion.[17] Use of recombinants between enteric and respiratory TGEV strains (attenuated TGEV) demonstrated that a substitution in aa 219 of the S protein was associated with loss of enteric tropism. PRCV spreads by the aerogenic route or via droplets such as SARS CoV. It further resembles SARS CoV in its pronounced tropism for the lung, replicating to titres of 10^7–10^8 $TCID_{50}$ and producing interstitial pneumonia affecting 5–60% of the lung.[13,18] Despite the invariable presence of lung lesions, many PRCV infections are clinically mild or subclinical, although PRCV strains with smaller deletions in the S gene (621 nt) and intact gene 3 reportedly produced more severe disease.[19,20]

Clinical signs of PRCV, like those reported for SARS, include fever and variable degrees of dyspnoea, polypnoea, anorexia and lethargy.[13,18] Coughing and rhinitis are less common. Also like SARS,[21] PRCV targets lung epithelial cells and alveolar macrophages.[13,18] Lung infection leads to interstitial pneumonia with bronchiolar infiltration of mononuclear cells, lymphohistiocytic exudates and epithelial cell necrosis. Transient viraemia occurs and PRCV also has been isolated from nasal swabs, tonsils and trachea. Like SARS CoV[1,22] PRCV replicates in undefined cells in the intestinal lamina propria unaccompanied by villous atrophy but unlike SARS PRCV infections result in limited faecal shedding and no diarrhoea. Recently faecal isolates of PRCV were found with minor (point mutations) but consistent genetic changes in the S gene compared to nasal isolates from the same pig.[23] These findings suggest the presence of CoV quasispecies in a host with some strains more adapted to the intestine, a corollary potentially applicable to the faecal shedding of SARS CoV.[1,22] It is notable that the more virulent TGEV infections have been displaced following the widespread dissemination of PRCV in Europe. PRCV can disappear from herds in summer and re-emerge in older pigs in the winter,[13,18] presumably circulating in pigs as subclinical infections in the summer; this fact is of interest in the study of SARS.

Several cofactors exacerbate PRCV or TGEV infections or shedding. Underlying disease or respiratory co-infections, dose and route of infection and immunosuppression (corticosteroids) are all potential cofactors related to the severity of SARS. These cofactors can also exacerbate the severity of TGEV or PRCV infections.[13] These cofactors may also be relevant in the super-spreader phenomenon seen in the SARS epidemic.

1 *Impact of route (aerosols) and dose on PRCV infections*. Studies of experimental inoculation of pigs with PRCV strains indicate that administration of PRCV by aerosol compared to the oronasal route, or in higher doses, resulted in higher virus titres shed and longer shedding.[12] Similarly in two other studies, high PRCV doses induced more severe respiratory disease. Pigs given $10^{8.5}$ $TCID_{50}$ of PRCV had more severe pneumonia and deaths than pigs exposed by contact[24] and higher intranasal doses of another PRCV strain (AR310) induced moderate respiratory disease whereas lower doses produced subclinical infections.[19] The analogy to SARS CoV is that the route and dose of exposure may modulate or enhance the clinical disease.[25]

2 *Impact of respiratory viral co-infections on PRCV infections*. Co-infections of SARS CoV and other respiratory pathogens such as human metapneumovirus, rhinovirus and chlamydia have been noted (Chapter 7). The interaction between PRCV and other respiratory viruses in pigs may therefore be pertinent. Hayes *et al*.[26] showed that sequential dual infections of pigs with the arterivirus (order *Nidovirales*, like CoV), PRRSV followed in 10 days by PRCV significantly enhanced lung lesions and reduced weight gains compared to each virus alone. The dual infections also led to more pigs shedding PRCV nasally for a prolonged period and, surprisingly, to faecal shedding of PRCV. The lung lesions observed resembled those in SARS victims.[21]

In another study, Van Reeth and Pensaert[27] inoculated pigs with PRCV followed in 2–3 days by swine influenza A virus (SIV). They found that SIV lung titres were reduced in the dually infected pigs compared to those that were singly infected, 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. This is relevant to the proposed treatment of SARS patients with IFN-α (Chapter 20).

3 *Impact of respiratory bacterial co-infections on PRCV infections*. Respiratory viral infections enhance the potential for bacteria to colonize the lower respiratory tract in animals and humans. The outer membrane of gram-negative bacteria contains endotoxin or lipopolysaccharide (LPS) which is released in the lungs during bacterial infection or potentially after antibiotic treatments such as those commonly used in SARS patients.[25] Bacterial LPS is a potent inducer of proiniflammatory cytokines. Van Reeth *et al*.[28] showed that pigs infected with PRCV followed by a subclinical dose of *E. coli* LPS within 24 hours developed enhanced fever and more severe respiratory disease compared to each agent alone. They concluded that the effects were probably mediated by the significantly enhanced levels of proinflammatory cytokines induced. Thus there is a need to examine both LPS and lung cytokine levels in SARS patients as possible mediators of the severity of SARS.

4 *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.[29] 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. Tsunemitsu *et al*.[30] reported a recrudescence of BCoV faecal shedding in one of four winter dysentery BCoV-infected cows treated with dexamethasone. Similarly, treatment of older pigs with dexamethasone prior to TGEV challenge led to profuse diarrhoea and reduced lymphoproliferative responses only in the treated pigs.[31] These data raise issues for corticosteroid treat-ment of SARS patients related to possible transient immunosuppression leading to enhanced respiratory disease or increased and prolonged CoV shedding. Alternatively, corticosteroid treatment may be beneficial in reducing proinflammatory cytokines if they play a major role in lung immunopathology.[29]

Group 1 feline CoV (FCoV) as models for systemic and persistent CoV infection

Historically, two types of FCoVs have been recognized: feline enteric CoV (FECoV) and FIPV. Current information suggests that the two viruses are biotypes of a prototype FCoV and that the FECoV which causes acute enteric infections in cats establishes persistent infection in some cats, evolving into the systemic virulent FIPV in 5–10% of cats.[5,6] The initial site of FCoV replication is in the pharyngeal, respiratory or intestinal epithelial cells.[7,32] Clinical signs include anorexia, lethargy and mild diarrhoea with villous atrophy in the jejunum and ileum in severe cases. The prolonged incubation period for FIP and its reactivation upon exposure to immunosuppressive viruses or corticosteroids suggested that FCoVs could cause chronic enteric infections in cats.[7,32] Recent reports of chronic faecal shedding and persistence of FCoV mRNA or antigen in blood, ileum, colon and rectum of FCoV-infected cats for prolonged periods (up to 7 months) confirm this scenario.[5]

A key pathogenetic event for development of FIP is productive infection of macrophages with cell-associated viraemia and systemic dissemination of virus.[7,32] Stress (immunosuppressive infections, cat density, transport to new environments) leading to immune suppression may trigger FIP in chronically infected cats, similar to its role in shipping fever CoV infections of cat-

tle. Two major forms of FIP are recognized: the effusive form with a fulminant course and death within weeks to months and the non-effusive form that progresses more slowly. The effusive form is characterized by fibrin-rich fluid accumulation in peritoneal, pleural, pericardial or renal spaces with fever, anorexia and weight loss. Non-effusive FIP involves pyogranulomatous lesions with thrombosis, CNS or ocular involvement. Fulminant FIP with accelerated early deaths appears to be immune-mediated in FCoV seropositive cats and can be enhanced by IgG antibodies to the S protein, although other contributing immune factors (inflammatory mediators such as cytokines, leukotrienes and prostaglandins initiated by C' activation or released by infected macrophages) may also play a role.[7,32] At least two mechanisms implicating IgG antibodies to FCoV S protein in FIP immunopathogenesis have been described. In the first, circulating immune complexes (IC) with C' depletion in sera and IC in lesions are evident in cats with terminal FIP.[7] For the second, antibody-dependent enhancement (ADE) of FCoV infection of macrophages has been described *in vitro* as mediated by neutralizing IgG MAbs to the S protein of FIPV, or of interest, to the antigenically related CoV, TGEV.[33] Similar accelerated disease was seen *in vivo* in cats inoculated with recombinant vaccinia virus expressing the S protein (but not the M or N proteins) of FIPV.[7,32] Thus the spectrum of disease evident for FCoV/FIPV exemplifies the impact of viral persistence and macrophage tropism on CoV disease progression and severity. The scenario of immune-enhanced disease is one to be kept in mind in the development of SARS vaccines.

Antigenic relationships and cross-species transmission: the example of group 1 coronaviruses

Within group 1 CoVs, TGEV, PRCV, CCoV and FCoV share close biological, antigenic and genetic relationships and they may represent host range mutants of an ancestral CoV.[13,34] The four CoVs cross-react in VN and IF tests and with MAbs to the S, N or M proteins (Table 12.1).[13] Duarte *et al*.[10] reported that the group I CoV PEDV is genetically more closely related to human CoV 229E than to TGEV, raising unanswered questions about its origin.

Both CCoV and FIPV infect baby pigs, but with only the latter virus causing diarrhoea and intestinal lesions similar to those caused by TGEV.[13] Cats infected with TGEV shed the virus in faeces and seroconverted to TGEV and FIPV;[13] cats exposed to CCoV remained clinically normal and did not shed the virus, but seroconverted to CCoV and FIPV.[32] Cats, dogs and foxes which seroconvert to TGEV were suggested as possible subclinical carriers of TGEV serving as reservoirs between seasonal (winter) epidemics, but only virus excreted by dogs was infectious for pigs.[13] Birds (*Sturnus vulgaris*) and flies (*Musca domestica*) have been proposed as mechanical vectors for TGEV.[13] These observations may be relevant to considerations of the ecology of the precursor animal SARS CoV.

Group 2 bovine CoVs (BCoV): models for pneumoenteric CoV infections

The shedding of SARS in faeces of most patients and the occurrence of diarrhoea in 10–27% of patients (Chapter 3)[25] suggests that SARS may be pneumoenteric like BCoV. BCoV (Fig. 12.1A) causes three distinct clinical syndromes in cattle: calf diarrhoea; winter dysentery with haemorrhagic diarrhoea in adults, and respiratory infections in cattle of various ages including cattle with shipping fever (Table 12.3).[14,35,38] Genetic differences (point mutations but not deletions) have been detected in the S gene between enteric and respiratory isolates, including ones from the same animal (Table 12.2).[36,36a] Unlike SARS and group 1 CoV, BCoV possesses a double layer of surface projections: the

Table 12.3 Summary of disease syndromes associated with BCoV infections

Disease syndrome	Clinical signs	Cells infected	Lesions[a]		Shedding[b]		
			Respiratory	Enteric	Nasal	Faecal	Ages affected
Calf diarrhoea	Diarrhoea Dehydration Fever/anorexia	Intestinal/ nasal/±lung epithelial cells	+/– lung emphysema	++ J, I, Colon Villous atrophy	2–8 days	2–8 days	Birth to 4 weeks
Winter dysentery	Haemorrhagic Diarrhoea Dehydration ±Rhinitis/dry cough Fever/anorexia	Intestinal/ nasal/±lung? epithelial cells	NR	++ J, I, Colon Enterocolitis	NR	1–4 days	6 months to adult
Calf pneumonia	Cough Rhinitis ±Pneumonia ±Diarrhoea Fever/anorexia	Nasal/±lung Tracheal ±Intestinal epithelial cells	+/– pneumonia	+/– J, I, Colon Villous atrophy	NR	NR	2 wks to 6 months
Shipping fever	Cough/dyspnoea ±Rhinitis ±Pneumonia ±Diarrhoea Fever/anorexia	Nasal/trachea Bronchi/alveoli ±Intestinal epithelial cells	Interstitial emphysema Bronchiolitis Alveolitis	+/– J, I, Colon Villous atrophy	5–10 days (17 days)	4–8 days (17 days)	6–10 months

[a]J = jejunum, I = ileum; NT = not tested.

[b]Shedding detected by infectivity or antigen assays; parentheses denote shedding detected by RT-PCR; NR = not reported.

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

shorter haemagglutinin (HE) and the longer spike (S), both of which function in viral attachment and fusion, induction of VN antibodies and immunity and haemagglutination of erythrocytes. Whether the HE influences the respiratory tropism or the broad host range of BCoV is unclear. Of interest, the HE of BCoV has homology with the HE of group C influenza viruses suggesting a prior recombination event between the two viruses.[8] Of concern is whether similar recombinants could arise between SARS CoV and influenza strains if co-infections were to occur in the future.

Calf diarrhoea and calf respiratory BCoV infections

Calf diarrhoea 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.[15,37] After an incubation period of 3–4 days, calves develop a severe, malabsorptive diarrhoea persisting for 3–8 days and resulting in dehydration and often death. Concurrent faecal and nasal shedding can occur. BCoV are also implicated as a cause of mild respiratory disease (coughing, rhinitis) or pneumonia in 2–24-month-old calves and are detected in nasal secretions, lung and often the intestines.[15]

More recent studies have implicated BCoV in association with respiratory disease (shipping fever) in feedlot cattle.[35,38] BCoV was isolated from nasal secretions and lungs of cattle with pneumonia[39] and from faeces.[39a] In a subsequent study, a high percentage of feedlot cattle (45%) shed BCoV both nasally and in faeces.[39a]

Shipping fever is recognized as a multifactorial, polymicrobial respiratory disease complex in feedlot cattle with several factors exacerbating respiratory disease, including BCoV infections as well as stress.[35,38,39,39a]

Group 2 BCoVs: cross-species transmission

The likelihood that SARS CoV is a zoonotic infection potentially transmitted from wild animals to humans is not surprising in light of previous research on interspecies transmission of BCoV including wildlife reservoirs. Although many CoVs have restricted host ranges, some such as BCoV appear to be promiscuous. In 1994, Zhang *et al.*[40] isolated a human enteric CoV from a child with acute diarrhoea (HECoV-4408) which was genetically (99% nt identity in the S and HE gene with BCoV) and antigenically more closely related to BCoV than to HCoV-OC43, suggesting this isolate is a BCoV variant. Tsunemitsu *et al.*[41] isolated CoV, antigenically closely related to BCoV by two-way cross-neutralization tests, from captive wild ruminants in Ohio, USA including Sambar deer (*Cervus unicolor*), white-tailed deer (*Odocoileus virginianus*) and a waterbuck (*Kobus ellipsiprymnus*) with bloody diarrhoea resembling winter dysentery in cattle. In addition, CoVs antigenically related to BCoV were isolated from elk and wapiti (*Cervus elephus*) in the western USA.[42] A more dramatic demonstration of the broad host range of BCoV was the experimental induction of infection and diarrhoea in SPF baby turkeys and contact controls, but not in chicks, with an enteric strain of BCoV[43] documenting CoV transmission from mammalian to avian species. More recent data suggest that CoVs genetically closely related to BCoV also occur in dogs with kennel cough.[44] Reasons for the broad host range of BCoV are unknown, but might relate to the presence of the HE on BCoV and its possible role in virus binding to diverse cell types.

Group 3 CoVs. Infectious bronchitis virus (IBV): model for respiratory CoV infection with other target tissues

IBV is a highly contagious respiratory

disease of chickens, like SARS, spread by aerosol or possibly faecal–oral transmission, and with a worldwide distribution.[45,46] Genetically and antigenically closely related CoV have been isolated from pheasants and turkeys,[47,48] but in young turkeys, they cause mainly enteritis. Respiratory infections of chickens are characterized by tracheal rales, coughing and sneezing with the disease most severe in chicks.[45,46] IBV also replicates in the oviduct causing decreased egg production or quality. Nephropathogenic strains cause mortality in young birds, whereas in broilers death ensues from systemic *E. coli* infections after IBV damage to the respiratory tract.

IBV replicates in epithelial cells of the trachea and bronchi, intestinal tract, oviduct and kidney, causing necrosis and oedema with small areas of pneumonia near large bronchi in the respiratory tract and interstitial nephritis in the kidney.[45,46] Of interest in SARS investigations is the persistence of IBV in the kidney and its prolonged faecal shedding since SARS CoV is detected in urine and shed longer term in faeces. Importantly, the respiratory tropism of one serotype of IBV was altered to kidney tropism by *in vivo* serial passage of virus via the cloacal route.[49] Both diagnosis and control of IBV are complicated by the existence of multiple serotypes and the occurrence of IBV recombinants.[45,46] This is unlike the scenario for most group one or two respiratory CoVs in which only one or two (FCoV) serotypes are known. Also relevant to SARS CoV is the finding that IBV strains also replicate in Vero cells, but only after passage in chicken embryo kidney cells.[45]

Animal respiratory or enteric CoVs: treatments and vaccines

Treatments with IFN-α

Interferons (IFNs) are of major interest for treatment of patients with SARS, but their potential effectiveness is unknown. Human recombinant IFN-α (rHuIFN-α) inhibited FIPV replication *in vitro*.[32] However, *in vivo* rHuIFN-α plus an immunomodulating drug failed to protect cats significantly against fatal FIPV disease, although the treatment suppressed clinical signs and prolonged survival time in cats.[50]

Similarly during a field outbreak of TGEV, 1–23-day-old pigs treated orally for 4 days with 1–20 IU of rHuIFN-α had significantly greater survival rates than had placebo pigs.[51] However, in piglets given rHuIFN-α shortly after birth, there was no increased survival. Thus *in vivo* treatment of CoV-infected animals with rHulFN-α produced variable results.

Animal CoV vaccines

1 *Enteric CoV vaccines*. Major efforts will probably be focused on development of SARS CoV vaccines. An understanding of the pathogenesis of SARS CoV infections including the target organs infected and how the virus is disseminated to these organs will assist in the development of effective vaccine strategies to block viral dissemination and protect the target organs. In monogastrics (pigs, humans) which secrete SIgA antibodies in milk, vaccination is accomplished by exploiting the common mucosal immune system. Because neutralizing SIgA antibodies to TGEV in milk are a correlate of protection to TGEV, the strategy is to evoke the gut-mammary IgA axis (first described in studies of immunity to TGEV; reviewed in ref. 13, 51a) by administering attenuated TGEV (TGEV-A) vaccines orally to induce SIgA antibodies in milk via intestinal stimulation of the mother. Problems[13] were encountered in the field application of this strategy such as poor titre and immunogenicity. Use of less attenuated TGEV strains or the antigenically related FIPV caused disease in baby pigs. These studies illustrate further the difficulty in priming for protective SIgA mucosal immune responses, even using live vaccines, in naïve

seronegative animals. However, in comparison, killed TGEV vaccines given parenterally (IM) induced only IgG antibodies in milk and no protection against TGEV. Interestingly, a single infection of the respiratory tract of pigs or sows with the TGEV deletion mutant, PRCV, induced only partial active or passive immunity to TGEV, respectively[13,52] but repeated PRCV infections of the mother induced higher IgA milk antibody responses and protection rates.[53] Van Cott *et al*.[52] found that in young pigs this was because a single PRCV infection of the respiratory tract induced few IgA antibody secreting cells (ASC) in the intestine, but higher numbers of IgG ASC in the lower respiratory tract (bronchial lymph nodes), and primed for anamnestic IgG and IgA intestinal antibody responses after TGEV challenge leading to the partial protection observed. 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.[13] SARS CoV frequently causes intestinal infections as well as pneumonia. Experience with animal coronavirus vaccines suggest that neither killed parenteral nor respiratory applied vaccines may prevent the diarrhoeal disease or faecal shedding.

Attempts have also been made to develop TGEV subunit vaccines to induce active immunity to TGEV in older pigs (Table 12.4).[54–56] It is likely that similar strategies may be devised for SARS CoV vaccines. Again problems were encountered in providing effective active immunity against TGEV-induced diarrhoea as summarized by the protection data in Table 12.4. Two subcutaneous doses of two different baculovirus-expressed S glycoprotein constructs containing the four major antigenic sites (including the immunodominant site A) elicited neutralizing antibodies in serum, but failed to induce any protection against

Table 12.4 Active immune responses and protection in pigs to recombinant TGEV spike (S) glycoprotein with or without the N and M proteins[a]

Inoculum[b] **(50 μg/dose)**	**Inoculation route**[c]	**Adjuvant**[d]	**VN Ab serum**[e]	**Intestinal (MLN)**[f] **ASC responses**	**Diarrhoea morbidity**
$S_{A–D}$ (1449 AA)	SC 2X	IFA	Yes	NT	100%
$S_{A–D}$ (789 AA)	SC 2X	IFA	Yes	NT	100%
S_{C+D} (397 AA)	SC 2X	IFA	No (PC)	NT	100%
					Faecal shedding
S + N + M	IP 2X	IFA + mLT R192G	Yes	IgA	38%
Killed TGEV (vaccine)	IP 2X	Undefined	Low	None	86%
TGEV-V	Oronasal	None	Yes	IgA/IgG	0%
Mock	IP 2X	IFA + mLT	No	None	100%

[a]Shoup *et al*., 1997;[55] Sestak *et al*., 1999.[54]

[b]Truncated forms of baculovirus expressed TGEV S glycoprotein included the four major antigenic sites (A–D) or only sites C + D were tested. For baculovirus expressed S + N + M, S (789 AA), N and M proteins were mixed and tested. TGEV-V = virulent TGEV; mock = control.

[c]SC = subcutaneous; IP = intraperitoneal.

[d]IFA = Incomplete Freund's adjuvant; mLT R192G = mutant *E. coli* heat labile toxin lacking toxicity; Undefined = commercial vaccine adjuvant.

[e]VN Ab = Neutralizing antibody; (PC) = post-challenge only.

[f]MLN = Mesenteric lymph node; NT = not tested.

TGEV-induced diarrhoea (Table 12.4).[55] However, neutralizing antibodies induced against the recombinant S protein with antigenic site A given passively (orally) to TGEV-challenged pigs delayed the onset of diarrhoea and virus shedding.[56] The data confirm earlier findings using killed TGEV vaccines, which indicated that serum neutralizing antibodies (in contrast to intestinal IgA antibodies) do not correlate with a high degree of protection against TGEV infection.[13] However, in a subsequent study, partial protection against TGEV infection (faecal shedding) was induced in pigs vaccinated intraperitoneally with the S glycoprotein mixed with the N and M proteins (Table 12.4).[54] 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.[57] Thus in spite of long-term research efforts, effective TGEV vaccines have remained elusive, but with the emergence of PRCV, nature appears to have generated its own highly effective vaccine for the more virulent TGEV infections.

2 *Respiratory CoV vaccines*. In spite of its economic impact, no respiratory CoV vaccines have been developed to prevent BcoV-associated pneumonia in calves or in cattle with shipping fever. The correlates of immunity to respiratory BCoV infections remain undefined. Limited data from epidemiological studies of BCoV infections in cattle suggest that serum antibody titres to BCoV may be a marker for respiratory protection. However, whether the serum antibodies are themselves correlates of respiratory protection or only reflect prior enteric or respiratory exposure to BCoV is uncertain.

The only available animal CoV vaccines targeted to prevent respiratory CoV infections are IBV vaccines for chickens. Both live attenuated and killed commercial IBV vaccines are used.[45,46] Attenuated vaccines are used in broilers, usually at 1 day of age and 10 days later, since only short-term (6–7 weeks) protection is needed. For layers or breeders where longer protection is needed (~18 months), attenuated vaccines are used for priming at 2–3 weeks of age followed by injection of killed oil–emulsion vaccines, often at 8–10-week intervals throughout the laying cycle. The correlates and mechanisms of protection against IBV clinical disease are uncertain but neutralizing antibody does appear to be relevant.[46] Evidence exists that the S1 glycoprotein, but not the N or M proteins of IBV can induce protection, although all three proteins induce cell-mediated immune responses to IBV.[58] Problems encountered in vaccine protection include the existence of multiple serotypes/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 back-passage in chickens[59] with the suggestion that point mutations in the genomes of attenuated vaccines may generate new epidemic strains of IBDV.[60] (See Chapter 22.)

3 *FIPV vaccines*. Because of the immunopathogenesis of FIPV, vaccines for its control have been among the most problematical to develop. Most conventional FIPV vaccine approaches (killed and attenuated) have failed and, in fact, they induced accelerated disease and reduced survival times.[7,32] These adverse effects have been attributed to antibodies to the S protein which can mediate immune complex disease or ADE of infection. A recombinant vaccinia virus expressing the S protein also mediated this effect. The efficacy of a commercially available temperature-sensitive FIPV vaccine is also debated, although there is no evidence that it causes accelerated FIP. Attempts to circumvent use of the S protein, by priming with DNA vaccines containing the M and N proteins (augmented by co-delivery of plasmids encoding feline IL12) and then boosting with recombinant vaccinia virus encoding the N and M proteins also failed and even enhanced susceptibility of the vaccinated cats to FIP.[61] Thus the development of safe and effective FIPV vaccines remains elusive.

Concluding remarks

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 co-infections (viruses, bacteria, LPS). These variables may also influence the severity of SARS and transmission of SARS. 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 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 small number of human and animal CoVs. In addition, interspecies transmission of certain CoVs may not be uncommon, although the determinants of host range specificity among CoVs are undefined. Early examples of CoVs with broad host range include TGEV, CCoV and FCoV which appear to be host-range mutants of an ancestral CoV. Even more promiscuous are BCoVs which cross-infect diverse species from wild ruminants to baby turkeys and appear as genetically similar strains in dogs and even humans. Thus it is not unprecedented for new CoV strains to emerge or for interspecies transmission of CoVs to occur. As a reminder of this potential disease threat, it is estimated that 75% of emerging human pathogens are zoonotic,[62] but we understand very little about CoVs or other viruses circulating in wildlife or their potential to emerge as either public or animal health threats.

Development of safe and efficacious vaccines for animal CoV infections has been problematic and only partially successful. Problems encountered 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 non-replicating 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.[13,51a] In addition, most vaccines for mucosal pathogens may fail to induce sterilizing immunity or prevent reinfections, as commonly observed for natural CoV mucosal infections, and the major vaccine focus may be to prevent severe disease. Although early studies of immunity to TGEV infections provided evidence for new immunologic linkages (gut–mammary axis and a common mucosal immune system, reviewed in ref 13, 51a), subsequent studies of TGEV/PRCV demonstrated compartmentalization within the respiratory and intestinal components of the common mucosal immune system, influencing the protection levels induced and future strategies for mucosal vaccines.[12,52]

An understanding of CoV disease pathogenesis is critical for the design of effective vaccine strategies. For SARS, many unanswered questions remain: these include the following. What is the initial site of viral replication and is SARS pneumoenteric like BCoV or primarily targeted to the lung like PRCV with faecal shedding of swallowed virus and with other sequelae contributing to the diarrhoea reported? Does SARS CoV infect the lung directly or via viraemia and does it productively infect secondary target organs (intestine, kidney) via viraemia after replication in lung? 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. But as illustrated for IBV, live vaccines may revert to virulent if inadequately attenuated, raising safety issues. Finally, the macrophage-tropic, systemic

FIPV CoV infection of cats presents yet another vaccine dilemma in that neutralizing IgG antibodies to FIPV, not only fail to protect, but actually potentiate the immunopathogenesis of FIPV.

In summary, although much progress has been made in the comparative biology of animal coronaviruses that is applicable to SARS CoV infections, much remains unknown as highlighted in this chapter. Perhaps the SARS epidemic will generate new interest in these fundamental research questions related not only to CoV infections, but also to other infectious diseases of humans and animals.

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