Coronaviruses with Special Emphasis on First Insights Concerning SARS
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Coronavirus infections in veterinary medicine

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Introduction

The evidence that the "SARS-virus" could be an animal coronavirus and/or could originate from an animal coronavirus has increased the level of interest for coronavirus infections in animals. It was reported during the outbreak of SARS that researchers from HongKong University examined 25 animals representing eight species in a live animal market in southern China and found the virus in six palm civets. The same investigators also found the "SARS-virus" in a badger and a raccoon dog (Yahoo on Friday, May 23rd; The Wall Street Journal, May 27th). It is noteworthy that food handlers represented more than 30% of the early SARS cases. It is still not clear yet whether the virus was transmitted from animals to humans or *vice versa*.

Coronavirus infections have been recognized as causative agents for deadly diseases or important economic factors for a long time (see Tab. 1).

Coronaviruses are distributed worldwide. Many coronaviruses are propagated in the respiratory or the intestinal tract and are accordingly transmitted by the aerogenic or by fecal-oral route. Most coronaviruses cause clinical signs in the infected animals.

A natural or artificial transmission into other species than the natural host has been described for many coronaviruses although the virus preferably spreads within one host species.

Coronaviruses and the toroviruses represent separate genera within the *Coronaviridae* family (see the chapter by Cavanagh in this book; [1–3]). The *Coronaviridae* are named after their relatively unique virion morphology. In addition, coronaviruses have unique genome features and a replication strategy that distinguishes them from other RNA viruses.

Together with the *Arteriviridae*, *Coronaviridae* belong to the order of *Nidovirales*. These viruses have plus-strand RNA genomes that are transcribed to yield a nested set of overlapping sub-genomic mRNAs with a common 3'end. One of the most intriguing aspects of coronavirus replica-

Table 1. Overview of coronaviruses most relevant in veterinary medicine: their natural hosts and associated diseases and clinical manifestations.

Virus	Host	Disease
Feline infectious peritonitis virus (FIPV, FCoV)	Cat	Peritonitis
Transmissible gastroenteritis virus of swine (TGEV)	Swine	Gastroenteritis
Avian infectious bronchitis virus (IBV)	Chicken	Bronchitis
Mouse hepatitis virus (MHV)	Mouse	Hepatitis
Canine enteric coronavirus (CECoV)	Dog	Gastroenteritis
Turkey bluecomb coronavirus (TCoV)	Turkey	"Bluecomb disease"
Bovine coronavirus (BCoV)	Cattle	Gastroenteritis Respiratory symptoms
Rat coronavirus	Rat	Respiratory symptoms
Sialodacryadenitis virus (SDAV)	Rat	Sialodacryadenitis
Porcine epizootic diarrhea virus (PEDV)	Swine	Gastroenteritis
Porcine haemagglutinating encephalomyelitis virus (HEV)	Swine	Encephalomyelitis
Porcine respiratory coronavirus (PRCoV)	Swine	Respiratory symptoms
Feline enteritic coronavirus (FCoV)	Cat	Gastroenteritis

tion is high frequency RNA recombination [4]. This strategy might be important for the crossing of species barriers for many important animal coronaviruses as well as the "SARS-CoV". The members of the *Nidovirales* order, however, especially differ with respect to their envelopes and nucleocapsids [5].

The biology of coronaviruses has been described in detail in other chapters. Therefore, this chapter focuses on clinical and economical aspects of coronavirus infections in companion (pets), live-stock (farm) and laboratory animals.

Based on phylogenetic analysis and antigenic cross reactivity, three groups can be distinguished in the *Coronaviridae* family. Some important viruses that are discussed below belong to group I and include the canine enteric coronavirus (CECoV), the transmissible gastroenteritis virus (TGEV) of swine, the porcine epidemic diarrhoea virus (PEDV), the porcine respiratory coronavirus (PRCoV) and the feline coronaviruses (FCoVs).

Most coronaviruses are enzootic and/or endemic in the respective host species. The vast majority of infections occur with inapparent clinical signs. The virus usually infects and replicates in epithelia and outbreaks of gastrointestinal and/or respiratory diseases are often seasonal. Clinical signs are in general more severe in younger animals. Immunocompromised animals and infected adults also serve as a major virus reservoir.

Genomic recombination appears to be a very common event *in vitro* and *in vivo* [6] in coronaviruses also relevant in animal health. Therefore, interactions of different coronaviruses can lead to new types and novel clinical entities [7, 8].

Specific infections

Coronavirus infections in swine

Porcine coronaviruses include i) TGEV; ii) PRCoV which is a mutant of TGEV; iii) PEDV I and II; and iv) haemagglutinating encephalomyelitis virus (HEV). The latter belongs to the coronavirus group II.

TGEV and PEDV are highly infectious and highly contagious enteric viruses of swine. PEDV and TGEV infections are considered difficult to distinguish clinically and also histopathologically [9–11] but can be distinguished by using modern, up-to-date techniques such as PCR and/or specific sequencing [12]. TGE is an economically important disease that might result in high mortality and was first described in the 1940s. While older animals generally recover by time, piglets under the age of three weeks usually die from the infection. The TGEV genome consists of a single-stranded, positive-sense 28.5-kb RNA. The viral membrane contains three transmembrane proteins: the S protein (220 kDa), M protein (29 to 36 kDa), and minor E protein (10-kDa). The S surface protein initiates the infection by binding to the cell surface. It also mediates the subsequent fusion between the viral and cellular membranes. The S protein binds to aminopeptidase N and to sialic acid. Aminopeptidase N binding is required for TGEV to initiate the infection of cells [13]. Recognition of sialic acids appears to be important for both, the haemagglutinating activity and the enteropathogenicity of TGEV [14].

The enteric tropism of TGEV presumably also requires the binding to a co-receptor that maps around amino acid 219 of the S protein as well as other additional co-factors [15, 16]. The importance of sialic acid binding for entropathogenicity is supported by the fact that the non-enteropathogenic PRCoV lacks sialic acid binding activity. This can be explained by a large deletion in the S gene that results in a truncated spike protein [17, 18]. PRCoV causes a mostly mild and moderate epizootic respiratory disease and might have worked like a vaccine against TGE in a number of swine populations.

TGEV is transmitted either directly or indirectly through contact with feces of infected pigs or *via* vector animals such as dogs and cats. Like other coronaviruses, TGEV is fragile and highly sensitive to disinfectants as further detailed in the chapter by Wolff et al. Epidemic peaks mostly occur during the cold season. After an incubation period of one to three days, symptoms of TGEV-associated disease emerge that include watery diarrhea, typical foul smelling yellowish-green feces that often contains flecks of undigested milk, vomiting, and loss of appetite. While the mortality rate is high in suckling piglets – up to 100% in piglets under two weeks of age – clinical symptoms in older piglets and adults are often mild and these animals will survive if especially their hydration status is adequate and ensured. The mortality rate is generally lower in these animals and will

largely depend on additional factors such as secondary bacterial infections, cardiovascular decompensation, chilling and dampness. Outbreaks usually only last a couple of weeks with occurrence of specific neutralizing antibodies in mucosa, blood and milk. Further, lactating sows are highly susceptible. Clinical signs may include vomiting, severe diarrhea, malnutrition, and cease of lactation. In large herds the disease can persist for some time, often contributing to post-weaning diarrhea. The clinical symptoms of endemic/enzootic TGE are usually less severe in the older pigs, making a clinical differentiation between TGE and other infectious enteric diseases, like that caused by rotaviruses and/or clostridia, impossible. Upon histopathological examination, villous atrophy is frequently found in both rotaviral and enzootic TGE infections. Mixed infections are possible and underscore the importance of a strict disease management and/or prevention regimen. TGE represents a reportable disease also in EU member states. There is no specific treatment available yet, however, electrolytes, nursing and enhanced management of the piglets may reduce mortality and lethality. In smaller herds cross-suckling of affected piglets onto recovered sows would offer a biological treatment. It is critical to almost impossible to assess general hygiene measures in prevalently infected herds. Modified live vaccines are available and immunization of pregnant swine is a common vaccination strategy.

Coronavirus infections in dogs

CECoV is associated with moderate to severe enteritis in young puppies. The genome contains the open reading frames (ORFs) 1a and 1b, encoding polyproteins leading to the viral replicase formation. Downstream of ORF 1b are ORFs encoding the coronavirus structural proteins S (ORF 2), E (ORF 4), M (ORF 5) and the nucleocapsid (N) protein [19]. The E protein has a function for virus assembly [20], the M protein is a type III glycoprotein [21]. ORF 2 encodes the spike (S) protein, a glycoprotein ranging from 1,160 to 1,452 amino acids (aa) in length [19]. This large protein has three structural domains. The large external domain at the N-terminus is furthermore organized into two sub-domains S1 and S2 with the S1 sub-domain including the N-terminal half of the molecule and forming the globular portion of the spikes. S1 contains sequences responsible for binding specific cellular receptors. S1 sequences are extremely variable, and mutations in the S1 region have been associated with problems of altered antigenicity and pathogenicity/virulence. In contrast, S2 sequences are genetically much more conserved and contain two heptad repeat motifs that suggest a coiled-coil structure [22].

Sequence analyses of CECoV detected in fecal samples that were collected from dogs with diarrhea showed mutations accumulating over the M gene [23]. A genetic drift to FCoV type II was also observed in the sequence

of CECoV detected in the faeces of puppies infected naturally during the late stages of long-term viral shedding. Infection by mixed populations of genetically different CECoV and recombination *in vivo* might, therefore, be common events [24].

The clinical signs of these coronavirus infections vary. They commonly include vomiting, diarrhea and "unspecific" symptoms such as depression, anorexia, and fever. Puppies most obviously die from severe dehydration. The majority of dogs that are not severely affected recover without any treatment. Animals with severe symptoms of dehydration need supportive care stabilizing the hydration status. Antibiotic/antibacterial treatment may be indicated in order to prevent exacerbations of bacterial superinfections. Although vaccination may be indicated in kennels, sanitation is also the economically most effective way to control these coronavirus infections and, therefore, should be maintained by keeping the kennels free of feces and cleaning the environment by an appropriate desinfection regimen.

Feline coronavirus infection

FCoV is commonly associated with mild enteric infections but is also associated with feline infectious peritonitis (FIP). FIP is a routinely fatal disease in both wild and domestic *Felidae*. FCoV can be distinguished into two serotypes: I and II, on the basis of a virus neutralization assay *in vitro* using both type-specific feline sera and monoclonal antibodies directed against the S protein [25–27]. The prevalence of these two serotypes is uncertain; type II FCoVs may account for up to thirty percent of the FIP cases in cats in Japan [26]. The S protein of the type II FCoV shares immunodominant neutralization epitopes with the S protein of canine coronavirus [25]. The S proteins of type II FCoV strains show great amino acid sequence identity to those of CECoV (approximately 91%) and TGEV (approximately 81%) but not to several type I strains (approximately 45%) [28, 29].

In the first phase of the infection the symptoms are extremely unspecific. A mild upper respiratory disease, as evidenced by watery eyes and sneezing, might be diagnosed. A high percentage of primarily infected cats clear the virus; some of them, however, become long-term virus carriers. Only a small percentage of exposed cats – higher, up to twenty percent in kennels – develop FIP, months or years after primary infection. It is still unclear whether an endogenous reactivation could also be responsible for this pathomechanism. The clinical signs of FIP usually gradually increase in severity over a period of several months, starting with rather unspecific signs such as inappetence, depression, rough fur, weight loss and fever. The forms of the lethal FIP may be effusive (wet) and/or non-effusive (dry and/or proliferative). Combinations of both clinical manifestations are

rather common. The most characteristic clinical sign of wet FIP is ascites. Other symptoms may be rather unspecific like swollen lymph nodes, ocular symptoms with conjunctivitis and/or corneal ulcers. As the name suggests, fluid accumulation is minimal in the dry form of FIP. Instead, other, rather unspecific symptoms dominate. The dry form progresses slowly, often making clinical diagnosis difficult. Weight loss, depression, anemia, and fever, are frequently observed symptoms. Signs of severe kidney and/or liver failure, pancreatic, neurological or ocular disorders are observed in various combinations. A characteristic granulomatous inflammation is mostly observed by biopsy or in pathological examinations if performed. A cure does not exist yet. The therapy should provide supportive care and to alleviate the self-destroying inflammatory response of the disease. Short-term remissions in a small percentage of patients have been described. A combination of corticosteroids, cytostatic drugs and antibiotics may be helpful in some cases despite the often fatal overall prognosis pro vitam.

Virulence of FCoV strains appears to correlate to their ability to infect macrophages [30]. The clinical symptoms are induced by immune complex reactions. Antibodies are not only not protective, they might even accelerate the onset and the course of the disease in form of an antibody-enhanced infection (AEI) such as observed in Dengue fever in humans [31]. The pathogenesis of the lesions, however, is not yet fully understood in its complexity. On the one hand there is evidence that immune complexes and subsequent activation of complement factors play an important role in the pathogenesis of FIP [32]. On the other hand, abnormal cytokine or chemokine secretion patterns – i.e. in infected immunocompetent cells – could also play a pathogenic role in the development of typical FIP lesions like in granulomas [5].

Bovine coronavirus infections and the possibility of transmission to humans

Bovine coronavirus (BCoV) is an important cause of neonatal calf diarrhea [33] but may also infect the respiratory tract and has been recognized as the causing agent especially for winter dysentery in adult cattle. Enteric and respiratory virus strains are antigenically related [34] but differ genetically [35]. Amino acid alterations in the S1 subunit of the S protein (e.g. residues 113, 115, 118, 146, 148, 501, 510 and 531) of respiratory isolates conferred significant changes to the structure of the protein compared with the BCoV strains that cause winter dysentery and calf diarrhea.

BCoV was first reported in 1972 [35–37]. The zoonotic potential of BCoV remains to be determined although a case of transmission to humans has been reported [38]. BCoV possesses a single-stranded, enveloped, nonsegmented RNA genome of positive polarity [39]. The mature virion con-

tains five major structural proteins – the nucleocapsid (N) protein, the transmembrane (M) protein, the haemagglutinin/esterase (HE) glycoprotein, the spike (S) protein, and a small membrane (E) protein [40, 41]. HE fulfills receptor binding and detachment functions. The S glycoprotein also recognizes the 9-O-acetylated sialic acid, apparently with a higher affinity than HE [42, 43]. The S protein is proposed to be responsible for the primary attachment of BCoV to other cell surface receptors [42]. Variations in the S glycoprotein are most likely responsible for host specificity and tissue tropism [44].

BCoV is distributed worldwide and antibodies can be detected in the vast majority of cattle [45, 46]. BCoV infects calves/cattle by both the oral and/or respiratory route. Although the virus can be detected in healthy animals, the most common source of enteric infection is diarrhoeic faeces from other infected animals. The virus infection of the enteritic tract starts in the small intestine and spreads after an initial replication throughout the gastrointestinal (GI) tract. Since the virus replicates in the surface distal villi of the epithelial cells of the GI tract, these cells will eventually be destroyed, leading to fusions of adjacent villi in the small intestine and to atrophy of the colonic ridges [33]. The severity of clinical signs varies with the age and especially the immunological status. Usually, a yellowish diarrhea is observed that lasts for about three to seven days. It is difficult to distinguish between rota- and coronavirus-associated infection based on clinical signs solely. If diarrhea is severe, calves become pyrexic and dehydrated.

Infections with respiratory BCoV often appear after stress such as shipment and/or environmental disturbances. Infected animals will develop clinical signs of respiratory distress including wheezing and nasal discharge three to four days after infection. Bacterial superinfections often complicate the clinical status. Respiratory disease was induced experimentally after oral inoculation in colostrum-deprived calves [47]. As for other coronaviruses, seasonal changes in temperature, environmental factors but also the immune status play an important role in the transmission of the virus and the clinical outcome of the infection. Different virus isolates have been reported to have differences in tissue tropism [48]. These authors report that about 50% of the infections in calves involve the respiratory tract in parallel and the enteric tract, whereas each 25% only involve either the respiratory or the GI tract.

As for other coronaviruses, diagnosis/diagnostics requires detection of specific nucleic acids. Virus isolation may be difficult and mostly not practicable in all day diagnostics. Alternatively, nasal swabs might be used for detection of BCoV antigen by immunofluorescence tests or other appropriate immunological methods.

Interestingly, a fragment amplified from “SARS-CoV” (BNI109 fragment) showed 75% homology with BCoV and mouse hepatitis virus (MHV) at the amino acid level [49].

Avian infectious bronchitis

Infectious bronchitis virus (IBV) is a major cause of disease in domestic fowl and causes an acute, highly contagious disease of the respiration and sometimes also urogenital tract [50]. The IBV genome consists of approximately 27 kb [51] and codes for the spike (S) glycoprotein, the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein [52]. IBV is distributed worldwide, and different variants have been isolated [53–58]. IBV strains within a geographic region might be unique and distinct, examples are Europe, the USA, and Australia, i.e. for avian IBV [59]. The different antigenic types make the use and possible efficacy of a single vaccine extremely questionable.

The natural hosts for IBV are chicken and pheasants. IBV infections represent an important economic threat for the poultry industry. Infected animals of all ages show signs of an acute, highly contagious respiratory disease. It is characterized by coughing, sneezing, and a nasal discharge. The major production loss results from the reduction in egg production and inferior egg quality. In younger birds there may be a high death rate, weight losses, or problems in weight development. Some virus strains may also induce primary infectious kidney lesions. The lethality in these animals may be up to 25%. As for other coronavirus infections, only a virological examination, mainly based on serological techniques, can lead to the appropriate diagnosis. Other important respiratory diseases include Newcastle disease (ND) and infectious laryngotracheitis (ILT). According to regulations of many EU member states, ND is a disease that requires immediate reporting and action by veterinary authorities. ILT is also a reportable animal disease. There is no specific treatment for infectious bronchitis. Antibiotic treatment might help to prevent or reduce secondary, i.e. bacterial superinfections. Strict hygienic management and/or isolation of the flock may help to interrupt the disease cycle. Different live virus vaccines have been developed and are currently in use; however, the use of live vaccines complicates especially serological diagnostics. Sequencing of the S1 glycoprotein gene is the method recommended by the OIE (Office International des Epizooties) to discriminate between different IBV strains.

Coronavirus infections in turkeys – "bluecomb disease"

Turkey coronavirus (TCoV) causes acute and highly contagious enteritis of significant economical importance in turkeys [60]. The clinical signs usually appear at seven to 30 days of age in turkeys under six weeks of age and consist of diarrhea, litter eating, decreased feed efficiency and decreased weight development. Morbidity is high, although mortality might be low. TCoV is difficult to eradicate. TCoV-induced enteritis has been described

to be Minnesota's most costly turkey disease from 1951 to 1971 [61]. TCoV treatments of the disease are often unsuccessful and there are currently no effective vaccines and/or other medications to prevent this disease. The local immune system of the mucosa of the gastrointestinal tract plays a major role in the protection against an infection and modulates clinical signs as well. Recent studies indicate that neutralizing, intestinal secretory mucosal IgA antibodies to TCoV are elicited in turkeys following infection with TCoV and that local mucosal antibodies may provide protective immunity for infected turkeys to recover from TCoV infection [62].

As for other coronaviruses, mechanical vectors play an important role in the transmission of the virus: it was demonstrated recently that house flies can transmit TCoV [63].

TCoV from intestinal contents of diarrheal poults could be propagated in a human adenocarcinoma line and one-day-old turkey poults inoculated orally with tissue culture-adapted TCoV isolates developed mild to severe diarrhea [64]. However, the passaging of TCoV could not be reproduced by other investigators so far [65, 66]. This viral enteritis is different from haemorrhagic enteritis (HE), another economically important disease of turkeys that is caused by a type II adenovirus (reviewed by Sharma 1991 [67]). In contrast to bluecomb disease, turkeys younger than four weeks of age are clinically "resistant" to HE. In addition to the enteritis, a pathological frequent finding is hepatosplenomegalia. Bursectomy and/or splenectomy abrogate clinical HE [68].

Coronavirus infections in mice – "mouse hepatitis"

MHV belongs to the *Coronaviridae* family and represents one of the most important pathogens of the laboratory mouse. MHV is serologically related to other coronaviruses of rats, pigs, cattle, and also humans. It is a very well-studied virus, because of its adverse influence on several research approaches and consecutively also results. About 25 different MHV strains have been reported so far. Some strains are polytropic; they infect a variety of tissues and cause symptoms in various organs. Other strains are more specifically organotropic, e.g. enterotropic, and cause villus attenuation, syncytia formation and mucosal necrosis of the terminal small intestine and the colon.

MHV is very contagious; transmission occurs by aerosol, faeces and many other contacts/transmission routes. There are usually no clinical symptoms in infected adult mice. Clinical signs such as weakness, diarrhea, wasting and weight loss are observed in young mice. The mortality rate varies but might be high. Since laboratory animals almost always come from controlled breeding providers/animal environment (e.g. SPF = specifically pathogen free), serology is a highly reliable method to detect the target infection.

Considering the costs of modern biomedical research, infection with MHV is an economically important disease in small laboratory animals. The effects of the MHV infection on immunocompromised mice include enhanced phagocytic activities of macrophages, rejection of xenograft tumors [69], abnormal tumor growth patterns [70], altered response to chemical carcinogens [71], and impaired liver regeneration. Pharmcokinetics of test compounds might also be altered due to a change of enzyme activities. In immunocompetent mice, effects observed include immunoestimulation and, later on, immunodepression [72, 73]. Macrophage function is altered in infected mice [74], and effects on other immune competent cells are frequently found in infected mice as well. Often the best solution for infected facilities is to eliminate the entire affected stock with a consecutive appropriate desinfection regimen.

Host range mutants of MHV strains were isolated from mixed cultures containing progressively increasing concentrations of non-permissive Syrian baby hamster kidney (BHK) cells. The mutant virus was polytrophic, replicating efficiently in normally non-permissive BHK cells, Syrian and Chinese hamster (DDT-1 and CHO) cells, human adenocarcinoma (HRT), primate kidney (VERO) and in murine 17Cl-1 cell lines [75].

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