Canine coronaviruses: emerging and re-emerging pathogens of dogs

Canine Coronaviren: Neu und erneut auftretende Pathogene des Hundes

Ahmed Abd El Wahed, Uwe Truyen

Canine coronavirus (CCoV) and canine respiratory coronavirus (CRCoV) are highly infectious viruses of dogs classified as Alphacoronavirus and Betacoronavirus, respectively. Both are examples for viruses causing emerging diseases since CCoV originated from a Feline coronavirus-like Alphacoronavirus and CRCoV from a Bovine coronavirus-like Betacoronavirus. In this review article, differences in the genetic organization of CCoV and CRCoV as well as their relation to other coronaviruses are discussed. Clinical pictures varying from an asymptomatic or mild unspecific disease, to respiratory or even an acute generalized illness are reported. The possible role of dogs in the spread of the Betacoronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is crucial to study as animal always played the role of establishing zoonotic diseases in the community.

Keywords: canine coronavirus, canine respiratory coronavirus, SARS-CoV-2, genetics, infectious diseases

Zusammenfassung


Schlüsselwörter: Canines Coronavirus, canines respiratorisches Coronavirus, SARS-CoV-2, Genetik, Infektionskrankheiten
**Introduction**

Canine coronaviruses are well known pathogens in dogs causing usual mild local disease affecting the gastrointestinal tract or the respiratory tract, but may also cause systemic infections (Decaro and Buonavoglia 2011).

At a closer look, the virus and the diseases are not as homogenous as it is suggested at a first glance. A number of very closely related and even one only distantly related virus comprise the “canine coronavirus” entity (Decaro and Buonavoglia 2008). Very recent reports of SARS-CoV-2 virus infections of dogs may even further broaden the spectrum of coronaviruses affecting this species (Sit et al. 2020).

**Genetics of canine coronaviruses**

Two types of canine coronaviruses are known: canine coronavirus (CCoV) and canine respiratory coronavirus (CRCoV). Both are member of the family Coronaviridae, which represent single-stranded, positive-sense RNA enveloped viruses (Figure 1).

Canine Coronavirus (CCoV) belongs to the sub-family Alphacoronavirus and CRCoV to Betacoronavirus (International Committee on Taxonomy of Viruses 2011). CCoV is more closely related to other Alphacoronavirus of cats (Feline coronavirus), pigs (Transmissible gastroenteritis virus, TGEV) and ferrets (Ferret coronavirus), while CRCoV is believed to have originated from the bovine coronavirus (BCoV) (Licitra et al. 2014). The CCoV genes are organized in the following order: replicase complex (open reading frame 1ab [ORF1ab]), spike (S) gene, ORF3abc, envelope (E) gene, membrane (M) gene, nucleocapsid (N) gene and ORF7ab (Decaro et al. 2015). The CCoV is subdivided into two distinct serotypes CCoV-I and CCoV-II (Le Poder 2011). The main differences between the two are present in the spike protein gene and, in addition, CCoV-I has a unique intact ORF 3 downstream of the S gene (Figure 2) (Le Poder 2011). The CCoV-II viruses can further be differentiated into -iia and -iib viruses, the latter being recombinant viruses between CCoV-II and TGEV (Ntafis et al. 2011).

The Alphacoronavirus of dogs, cats, and pigs are very closely related and because of their high genetic homology, they are considered by taxonomists as one virus species (Alluwaimi et al. 2020). Recombination of genetically very similar viruses has been described for various virus genera and also for Alphacoronavirus. Not only recombinants between the two CCoV genotypes were reported, but also viruses recombinant between pig and dog viruses as well as between cat and dog viruses (Licitra et al. 2014).

The genome of the CRCoV contains ORF1ab, non-structural (NS) protein, hemagglutinin-esterase (HE), S, NS protein, E, M, N, and internal N protein (I) gene sequences with similarities of more than 95% to the BCoV (Lim et al. 2013). The origin of CRCoV is unknown, but the virus most likely derived from the closely related BCoV. Experimental infections of puppies with BCoV support this hypothesis (Buonavoglia et al. 2006). CRCoV is also closely related to the human coronaviruses HCoV-OC43 and the human enteric coronavirus HECOv, which are all believed to be of bovine origin (Kin et al. 2016, Lu et al. 2017). Although sequence data of CRCoV are sparse, genetic recombination between BCoV and CRCoV has been reported particularly in orf1ab, M and N genes (Lu et al. 2017).

**Disease caused by canine coronaviruses**

CCoV causes usually a mild and self-limiting enteritis of young dogs under 12 weeks of age, or may even be subclinical (Licitra et al. 2014). The virus replicates in the intestinal epithelial cells of the villi in the small intestine, generally without causing too many lesions. However, a coinfection of CCoV and other pathogens, such as distemper virus, canine adenovirus or canine...
parvovirus (CPV) may enhance the disease severity (Alves et al. 2018). Particularly the co-infection with CPV is well known to lead to very severe clinical outcome with a high mortality (Pratelli et al. 1999b). While CCoV replicates in the epithelial cells of the villi in the small intestine, CPV propagates in small intestine in the actively dividing cells of the intestinal crypts, which are responsible for the generation of the epithelial cells (Zappulli et al. 2020). Regeneration of these cells, if damaged or killed by CCoV, leads to an increased division of the intestinal crypt cells and hence to a promotion of parvovirus replication and disease progression.

In recent years, an emerging type of CCoV-IIa was found to be able to produce a generalized form of the diseases similar to Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) (Decaro and Buonavoglia 2011). CCoV-IIa RNA could be detected in several intestinal organs as well as in the lung and brain of severely affected dogs. Because of its potential to cause a systemic infection, it was named pantropic CCoV. Some of the dogs were co-infected with parvovirus, which may contribute to the disease syndrome (Alfano et al. 2020). A consistent and characteristic clinical sign of infection with pantropic CCoV-II virus related disease is leukopenia (Decaro and Buonavoglia 2008). In a European survey, CCoV-IIa viruses were detected in extraintestinal tissues, and therefore assumed to be pantropic viruses, in dogs from France, Italy, Hungary, Greece, the Netherlands and Belgium (Decaro et al. 2013). Most of these viruses displayed a high homology within the ORF3 gene (Decaro et al. 2007).

Dry cough and nasal discharge were reported during infection with CRCoV. The virus has preferences for the nasal cavity, nasal tonsil, and trachea, but is rarely detected in lung, bronchial lymph nodes, and palatine tonsil (Erles and Brownlie 2008, Mitchell et al. 2009). CRCoV is a pathogen of the group of viral and bacterial pathogens of the canine infectious respiratory disease complex (CRID), or commonly called “kennel cough” (Priestnall et al. 2014, Wille et al. 2020). It is considered to cause a local infection, restricted to the respiratory system, however, in some naturally infected dogs, CRCoV was identified in spleen, mesenteric lymph nodes, and colon, but not in the enteric content (Erles and Brownlie 2008). In contrast to CCoV, CRCoV mainly infects dogs older than two years (Knesl et al. 2009).

**Geographical distribution**

CCoV was first described in a military dog unit in Germany in 1971 (Binn et al. 1974), thereafter, it became enzootic and has been identified worldwide. The pantropic variant was first described in Italy in 2005 (Buonavoglia et al. 2006), and sporadic cases have since been reported in various European countries (Decaro et al. 2013). The significance of this “new” disease manifestation, however, is still unclear.

**TABLE 1:** List of canine coronavirus vaccines (commercial names are available in https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/productsummaries/Vet-Labels-Category-Result?filters=Tax_Product-Summaries/animal-species/Canine

<table>
<thead>
<tr>
<th>Type</th>
<th>Form</th>
<th>Additional pathogen/s</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent</td>
<td>Killed</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Live-attenuated</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Divalent</td>
<td>Live-attenuated</td>
<td>Canine Parvovirus</td>
<td>–</td>
</tr>
<tr>
<td>Polyvalent</td>
<td>Live-attenuated</td>
<td>Canine Distemper-Adenovirus Type 2- Parainfluenza-Parvovirus</td>
<td>Canarypox</td>
</tr>
<tr>
<td></td>
<td>Killed CCoV and the rest is live-attenuated</td>
<td>Canine Distemper-Adenovirus Type 2- Parainfluenza-Parvovirus</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Killed CCoV</td>
<td>Leptospira Canicola-Grippotyphosa-Icterohaemorrhagiae-Pomona Bacterial Extract</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Killed CCoV and other viruses live-attenuated</td>
<td>Canine Distemper-Adenovirus Type 2- Parainfluenza-Parvovirus, Leptospira Canicola-Grippotyphosa-Icterohaemorrhagiae-Pomona Bacterial Extract</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Live-attenuated</td>
<td>Canine Distemper-Adenovirus Type 2- Parainfluenza-Parvovirus Vaccine, Leptospira Canicola-Icterohaemorrhagiae Bacterin</td>
<td>Canarypox</td>
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CRCov is considered an emerging infection, since it was first detected in the UK in 2003 (Erles et al. 2003). A retrospective study revealed the identification of the oldest cases of CRCov in Canada in 1996 (Ellis et al. 2005). Many European countries, United States and Japan have reported CRCov cases, while no records from other countries exist (Erles and Brownlie 2008, Mitchell et al. 2017).

**Diagnosis**

Diagnosis of coronavirus in general is difficult for several reasons. Cultivation of the virus in tissue culture is only possible for some serotypes in specific cells and some types like CCoV-I and CRCoV are particularly challenging (Erles et al. 2007, Regan et al. 2012). The virus displays a high mutation rate and cross-species transmission regularly occurs (Graham and Baric 2010). Nested PCR assays are highly sensitive and can detect minimal amounts of viral nucleic acid, but are very error-prone and susceptible for contamination (Pratelli et al. 1999a). Real-time reverse transcription-PCR assays were established for the detection of both CCoV and CRCoV (Decaro et al. 2004, Mitchell et al. 2009). Since the cross-reactivity to other related coronaviruses in PCR is remarkable, sequencing is considered one of the most effective techniques for the identification of canine coronavirus and its emerging types (He et al. 2020).

So far, only one antigen detection test for the identification canine coronavirus has been developed (Yoon et al. 2018). For antibody detection, specific CCoV and CRCoV tests are commercially available mainly for the N and S proteins (ELISAKITS 2021, Palmer-Densmore et al. 1998). In many occasions, the BCoV tests were used for the detection of antibodies to CRCoV because of their high similarities (Erles and Brownlie 2008, More et al. 2020).

**Treatment and prevention**

No specific antiviral drug is available for the treatment of canine coronaviruses. Many CCoV-II vaccines are commercialized and provide a good protection (Table 1), but they are less effective for CCoV-I and for CRCoV (Tizard 2020). One vaccine against CRCoV was on trial in France, but did not reach the commercialization stage (Shields and Abdelmagid 2013).

**SARS-CoV-2 and dogs**

The identification of SARS-CoV-2 in the nasal and oral cavities of asymptomatic Pomeranian and German shepherd dogs (2 out of 15 dogs tested), which lived in close contact with infected human cases in Hong Kong has opened the mind for the possibility of transmission of SARS-CoV-2 between humans and dogs (Sit et al. 2020). In the region of origin of SARS-CoV-2, in Wuhan, China, SARS-CoV-2 antibodies were detected in companion animals (2/10 cats and 1/9 dogs [Chen et al. 2020] and 16/946 dogs [Zhao et al. 2021]). In an independent study from Italy, a country hardly struck by COVID-19, 3.3% (n=314) of screened dogs were recorded positive for SARS-CoV-2 antibody, nourishing the fear of the dog playing a role in the epidemiology of the human pandemic (Patterson et al. 2020). A small surveillance study in France revealed a sero-positivity ratio of 58.8% in cats (20/34) and 38.5% in dogs (5/13) (Fritz et al. 2021). Despite a lot of serological evidence, all these reports must be taken with caution, since the serological assays for coronavirus always exhibit high cross-reactivity (Choy 2020, Hicks et al. 2021, Shrock et al. 2020). On the other hand, results based on virus isolation, PCR and sequencing revealed a more accurate analysis. Most of the identified COVID-19 dog cases were asymptomatic (Chen et al. 2020, Patterson et al. 2020, Sit et al. 2020). Only one case in a dog with transient respiratory distress and lethargy was recorded (de Morais et al. 2020). In contrary, cats showed a broad spectrum of clinical symptoms ranging from diarrhea, vomiting, sneezing and nasal discharge to dyspnea upon SARS-CoV-2 infection (de Morais et al. 2020). The dogs harbor in the respiratory tract only low levels of the target host receptor of SARS-CoV-2 (angiotensin-converting enzyme-2, ACE-2), while, in cats, this receptor is more abundant and genetically closer to the human ACE-2 (Sharun et al. 2020, Zhai et al. 2020). The similarities between SARS-CoV-2 and the canine coronaviruses (CCoV and CRCoV) is only approximately 45% (Sharun et al. 2020). Interestingly however, the S protein of the Betacoronavirus, HCoV-OC43 and CRCoV showed a homology of around 97% (Kim et al. 2016).

**Conclusions**

Coronaviruses have crossed the species barrier over the last few decades and continuous exposure of a new host to these viruses may promote the emergence of new strains or their adaptation to a “new” host. CCoV and CRCoV are outstanding examples of emerging diseases and for genetic recombination between members of Alphacoronavirus and Betacoronavirus, respectively. Understanding the evolution process of these viruses will provide us with knowledge to fight COVID-19 as well as to prevent future pandemic outbreaks.

**Ethical approval**

Not applicable.

**Conflict of interest**

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**Authors contribution**

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