Artykuł przeglądowy *Review*

Enteric and respiratory canine coronaviruses: importance and prevalence in Austria

KARIN MÖSTL

Institute of Virology, Department for Pathobiology, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

Received 08.04.2014 *Accepted 12.05.2014*

Möstl K.

Enteric and respiratory canine coronaviruses: importance and prevalence in Austria

Summary

A short introduction is given on canine enteric (CECoV) and canine respiratory coronaviruses (CRCoV), members of the genera Alphacoronavirus and Betacoronavirus, respectively. As in other European countries both viruses also circulate in Austrian dog populations. The clinical importance of CECoV has already been demonstrated. In the case of CRCoV, current knowledge of its clinical importance is limited, but CRCoV may be assumed to contribute to canine infectious respiratory disease. Diagnostic investigations, especially of CECoV, may be indicated. Because of the genetic variability of coronaviruses and the possibility of interspecies transmission events, further epidemiological investigations are needed.

Keywords: coronaviruses, dog, enteric, respiratory, epidemiology, Austria

Coronaviruses

The subfamily *Coronavirinae* of the family *Coronaviridae* includes many pathogens affecting humans as well as animals. They are responsible for enteric and/or respiratory disease, some of them also with generalizing and fatal outcome (e.g. feline infectious peritonitis).

Coronaviruses are large, enveloped, positive-stranded RNA viruses with a genome of 27,000 to 31,000 bases. The structural proteins are named S (spike)-, M (membrane)-, N (nucleocapsid)- and E (envelope)--protein. The S-gene encodes a large glycoprotein, which protrudes from the viral surface. It is important for the species specificity, virulence, and tissue tropism of the virus. The M-gene codes for the membrane protein, which is a transmembrane glycoprotein interacting with the nucleocapsid. The N protein is important for viral RNA synthesis. Betacoronaviruses contain an additional structural protein (HE) with haemagglutinin--esterase activity.

Tab. 1. Important members of the genera *Alpha-* and *Betacoronavirus* of the subfamily *Coronavirinae*, family *Coronaviridae*

Genus	Important members
Alphacoronavirus	Transmissible gastroenteritis virus (TGEV) Feline coronavirus (FCoV)/feline infectious peritonitis virus Canine enteric coronavirus (CECoV) Porcine respiratory coronavirus (PRCoV) Porcine epidemic diarrhoea virus (PEDV) Human coronaviruses 229E and NL63
Betacoronavirus	Bovine coronavirus (BCoV) Canine respiratory coronavirus (CRCoV) Porcine haemagglutinating encephalomyelitis virus (PHEV) Mouse hepatitis virus (MHV) Rat coronavirus Human coronaviruses OC43, HKU1 and 4408 SARS-CoV

Of special importance is the fact that the genome of coronaviruses is very variable; not only with respect to mutations, but also for recombination events between species-specific coronaviruses. For example, such recombinants were described for coronaviruses of carnivores (16, 28).

The subfamily *Coronavirinae* is actually divided into four genera: *Alpha-*, *Beta-*, *Gamma-*, and *Deltacoronavirus* (17). Tab. 1 shows important members of Alpha- and Betacoronaviruses. According to current knowledge, dogs may be affected by the canine enteric coronavirus (CECoV, member of the genus *Alphacoronavirus*) and the canine respiratory coronavirus (CRCoV, member of the genus *Betacoronavirus*). CECoV, transmissible gastroenteritis virus (TGEV), and feline coronavirus (FCoV) are very closely related genetically. Therefore they are regarded as host range variants rather than separate viruses (e.g. 9).

Canine enteric coronavirus (CECoV)

CECoV was first isolated in 1971 by Binn et al. (2) from faecal samples from military dogs with diarrhoea in Germany. CECoV was described as a pathogen for dogs of any breed and age, transmitted by the faecal--oral route and affecting the gastro-intestinal tract, usually without systemic generalisation and causing a broad spectrum of clinical courses. The infection may remain subclinical or be accompanied by mild gastroenteric symptoms with vomiting, diarrhoea, depression, and inappetence (2, 18). However, it may also take a fatal course, especially in young puppies (5) and in cases of double infection with canine parvovirus (CPV)-2 (27), although Evermann et al. (15) reported two cases of fatal CECoV-associated disease without evidence of co-infection with CPV-2. In mild cases, recovery of diseased dogs occurs spontaneously, and virus shedding continues for three to 16 days after infection (18).

Atypical canine coronaviruses were described in Italy by Pratelli et al. (24, 26, 28). These viruses show variations in the M-gene, which exhibits close similarity to feline coronavirus (FCoV). Therefore these variants were also called "FCoV-like" canine coronavirus. They were detected predominantly in shelters and kennels, and were assumed to appear by recombination events between FCoV and CCoV. Such recombinations are promoted by the fact that shelter conditions increase the likelihood of double infections with FCoV and CCoV, and that prolonged infection and the shedding of CCoV may occur in dogs in a similar way as in cats. Pratelli et al. (24, 26) showed that virus shedding may continue for several months after recovery. Infections with atypical canine coronaviruses are characterised by severe disease with bloody diarrhoea, as in CPV-2--induced disease, and high mortality.

Because of the similarity of the S-gene of CECoV to the S-gene of FCoV, CECoV were divided into two different genotypes: CECoV type I, whose S-gene is genetically very similar to that of FCoV type I, and CECoV type II, similar to FCoV type II (22, 25). Decaro et al. (10) established a PCR capable of discriminating between the two CCoV genotypes. Both genotypes could be detected simultaneously in faecal samples (9, 10, 22). Erles and Brownlie (12) and Decaro et al. (8) described new CCoV-II strains, which might have originated from a double recombination event with TGEV. Decaro et al. (9) identified 16% of CCoV-positive samples from diarrhoeic dogs from various European countries positive for this new variant, designated as CCoV-IIb (*versus* the classical CCoV-IIa). Ntafis et al. (21) reported even triple infections with CCoV-I/CCoV-IIa/CCoV-IIb in Greek dogs with diarrhoea.

A hypervirulent variant of CCoV-IIa was described in Italy by Buonavoglia et al. (3). It was named canine pantropic coronavirus, as it is characterised by spreading into internal organs and eventually causing fatal disease. Affected dogs show fever, inappetence, lethargy, haemorrhagic diarrhoea, severe leukopenia, and neurological signs. CCoV can be detected in various internal organs, indicating systemic distribution. The occurrence of these viruses has been reported in several European countries. Decaro et al. (6) investigated 354 carcasses of dogs that had died of systemic disease and had shown fever, leukopenia, depression, enteritis, respiratory disease, and/or neurological signs. The samples were collected from France, Hungary, Italy, Greece, the Netherlands, Belgium, and Bulgaria (only one sample) during the years 2009-2011. In total, 124 dogs were positive for CCoV, 24 of them with CCoV-IIa. This type was detected in all countries investigated with the exception of Bulgaria, from which only one sample was available. However, CPV-2 was also frequently identified in these carcasses. Therefore the authors point out that no definitive association between CCoV-IIa infection and the death of the infected animals can be established.

CECoV infections are widespread in dog populations. High seroprevalences are reported from European countries. For example, in Italy up to approx. 90% of dogs were reported to be seropositive (23, 30). In general, the infection risk is assumed to be greater for dogs in shelters and kennels. Stavisky et al. (33) report that CECoV can be shed at a high prevalence in kennelled dog populations, eventually leading to frequent transmission events. Various studies point to a considerable contribution of CECoV to gastro-enteric disease in dogs. Decaro et al. (9) reported the following percentages of CCoV-positive faecal samples from dogs with gastro-enteric disease: Spain 6%, the United Kingdom 27%, Portugal 36%, Italy 43%, Greece 56%, and Hungary 78%.

CECoV infections are also widespread in Austrian dog populations. The seroprevalence was first studied by Möstl et al. (20) in 48 dogs with and in 194 dogs without enteric disease. They reported 60.4% seropositive dogs with clinical signs of enteric disease and 69.6% of seropositive dogs without enteric disease. The seroprevalence against CECoV in Austria was reassessed on the basis of 127 blood samples randomly collected during the years 2003-2005 (32). These authors reported 88.2% seropositive dogs. No statistical differences were detected for parameters such as age, breed or sex. The authors also looked into the role of CECoV in dogs with enteric disease. While no single CECoV--positive faecal sample was obtained from dogs without enteric disease, 31.3% of dogs with gastro-enteric signs were positive for CECoV-specific nucleic acids in their faeces. CECoV-positive dogs were significantly younger than CECoV-negative ones. Dogs shedding high amounts of CECoV-specific RNA were younger than 12 months. Additional testing for CPV-2-specific nucleic acids revealed 40.6% of positive samples for dogs without enteric disease (all of them with high

ct-levels in realtime-PCR indicating low amounts of viral RNA) *versus* 87.5% of positive samples for diseased dogs. A high percentage of CECoV-positive animals also tested positive for CPV-2. Especially those that most probably shed CPV-2 intensively (as indicated by low ct-values), showed co-infection with CECoV. Such double infections are well known and reported by various authors (e.g. 27).

The atypical variant of CECoV has been detected in a large animal shelter in the south of Austria (1, 31). Faecal samples from 22 dogs with severe diarrhoea were investigated for CECoV-specific nucleic acids. Seventeen of them were CECoV-positive, and 11 positive dog samples were assigned to the "FCoV-like CCoV" according to typing in the M-gene region. Dogs shedding the variant tended to have stayed longer in the shelter, whereas all animals shedding typical CCoV had been in the shelter for only a few days. This fact seems to suggest that in shelters, where indirect contact between cats and dogs is possible, interspecies transmission of feline and canine coronaviruses may occur frequently, eventually leading to recombinant variants.

Only recently, CCoV-IIa was identified in Austria in various internal organs from puppies that had died at the age of only few days. This fact confirms the occurrence of pantropic CCoV in Austria (manuscript in preparation).

A vaccine against CCoV-induced enteric disease is commercially available. This vaccine is considered a "non-core" component (not obligatory for all dogs). Inactivated CCoV vaccines are believed to provide only incomplete protection. Moreover, the level of protection against CCoV variants has not been conclusively determined. According to Decaro et al. (7) immunity acquired through natural exposure to enteric CCoV did not fully protect against a pantropic CCoV strain.

Canine respiratory coronavirus (CRCoV)

CRCoV, a member of the genus *Betacoronavirus*, was described by Erles et al. (14) in dogs from a kennel with a history of endemic respiratory disease. CRCoV is antigenetically closely related to other members of *Betacoronaviruses*, namely bovine coronavirus (BCoV) and human coronavirus OC43. A recent common ancestor for these viruses and a bovine origin of CRCoV are assumed. The relationship between CRCoV and BCoV is used for diagnostic purposes, since BCoV may act as antigen in serological investigations for antibodies against CRCoV. The genetic similarity to CECoV is low (14).

CRCoV-specific nucleic acids can be detected in tracheal tissue samples from dogs with signs of respiratory disease than in clinically healthy animals. It is thought that CRCoV may be one of the aetiological agents contributing to canine infectious respiratory disease (CIRD; 4, 11, 13). Erles and Brownlie (11) and Buonavoglia and Martella (4) suggest that CRCoV infection is capable of facilitating the attachment of other pathogens or inhibiting the mucociliary clearance.

Dogs of any age and breed may be affected by CRCoV infection. Serological investigations indicate that seropositivity rates increase in older dogs (29, 30). CRCoV is assumed to contribute to clinical signs typical of CIRD, such as nasal discharge, coughing, and depression, eventually also bronchopneumonia and fever. Recently, Mitchell et al. (19) were able to reproduce such clinical signs by experimentally infecting dogs with CRCoV isolates.

Epidemiological investigations revealed seroprevalence rates of 59% in Canada, 55% in the United States, 30% in Ireland, and 36% in the United Kingdom (29). In Italy, Priestnall et al. (30) reported 23% of adult domestic dogs seropositive for CRCoV. Detection of CRCoV-specific nucleic acids in conjunctival, nasal or pharyngeal swab samples from dogs with respiratory signs was successful in up to 27% in tracheal swab samples from the United Kingdom (14).

In Austria, the first investigations for the presence of CRCoV in Austrian dog populations were performed by Spiss et al. (32). These authors reported a seroprevalence of 61.2% among 129 sera, which had been randomly collected during the years 2003-2005. Their study did not reveal any significant influence of age, sex or breed on serological results. CRCoV-specific nucleic acids were detected by PCR in three out of 34 swab samples from dogs with respiratory disease.

In conclusion, CECoV as well as CRCoV circulate in dog populations in Austria, as in many other European countries. Clinical importance has been clearly demonstrated for the enteric CECoV and may also be assumed for the respiratory CRCoV. Therefore, especially CECoV should be considered in diagnostic investigations. Owing to their genetic variability, coronaviruses exhibit a high potential for further evolution. Additionally, the possibility of interspecies transmission of these viruses increases the likelihood of recombination events, which have to be taken into account. Especially in multi-cat/dog environments (e.g. shelters) recombination events may play an important role.

References

1. *Benetka V., Kolodziejek J., Walk K., Rennhofer M., Möstl K.*: M gene analysis of atypical strains of feline and canine coronavirus circulating in an Austrian animal shelter. Vet. Rec. 2006, 159, 170-175.
2. *Binn L. N., Lazar E. C., Keenan K. P., Huxsoll D. L., Marchwicki R. H., Strano A. J.*: Recovery and characterization of a coronavirus from military dogs with diarrhea. Proc. Of the Annual Meeting of the United States Animal Health Association 1974, 78, p. 359-366.
3. *Buonavoglia C., Decaro N., Martella V., Elia G., Campolo M., Desario C., Castagnaro M., Tempesta M.*: Canine coronavirus highly pathogenic for dogs. Emerg. Infect. Dis. 2006, 12, 492-494.
4. *Buonavoglia C., Martella V.*: Canine respiratory viruses. Vet. Res. 2007, 38, 355-373.
5. *Carmichael L. E., Binn L. N.*: New enteric viruses in the dog. Adv. Vet. Sci. Comp. Med. 1981, 25, 1-37.
6. *Decaro N., Cordonnier N., Demeter Z., Egberink H., Elia G., Grellet A., Le Poder S., Mari V., Martella V., Ntafis V., von Reitzenstein M., Rottier P. J., Rusvai M., Shields S., Xylouri E., Xu Z., Buonavoglia C.*: European surveillance for Pantropic Canine Coronavirus. J. Clin. Microbiol. 2013, 51, 83-88.

7. *Decaro N., Elia G., Martella V., Campolo M., Mari V., Desario C., Lucente M. S., Lorusso E., Kanellos T., Gibbons R. H., Buonavoglia C.*: Immunity after natural exposure to enteric canine coronavirus does not provide complete protection against infection with the new pantropic CB/05 strain. Vaccine 2010, 28, 724-729.
8. *Decaro N., Mari V., Campolo M., Lorusso A., Camero M., Elia G., Martella V., Cordioli P., Enjuanes L., Buonavoglia C.*: Recombinant canine coronaviruses related to transmissible gastroenteritis virus of swine are circulating in dogs. J. Virol. 2009, 83, 1532-1537.
9. *Decaro N., Mari V., Elia G., Addie D. D., Camero M., Lucente M. S., Martella V., Buonavoglia C.*: Recombinant canine coronaviruses in dogs, Europe. Emerg. Inf. Dis. 2010, 16, 41-47.
10. *Decaro N., Martella V., Ricci D., Elia G., Desario C., Campolo M., Cavaliere N., Di Trani L., Tempesta M., Buonavoglia C.*: Genotype-specific fluorogenic RT-PCR assays for the detection and quantitation of canine coronavirus type I and type II RNA in faecal samples of dogs. J. Virol. Methods 2005, 130, 72-78.
11. *Erles K., Brownlie J.*: Canine respiratory coronavirus: an emerging pathogen in the canine infectious respiratory disease complex. Vet. Clin. North Am.: Small Anim. Pract. 2008, 38, 815-825.
12. *Erles K., Brownlie J.*: Sequence analysis of divergent canine coronavirus strains present in a UK dog population. Virus Res. 2009, 141, 21-25.
13. *Erles K., Dubovi E. J., Brooks H. W., Brownlie J.*: Longitudinal study of viruses associated with canine infectious respiratory disease. J. Clin. Microbiol. 2004, 42, 4524-4529.
14. *Erles K., Toomey C., Brooks H. W., Brownlie J.*: Detection of a group 2 coronavirus in dogs with canine infectious respiratory disease. Virology 2003, 310, 216-223.
15. *Evermann J. F., Abbott J. R., Han S.*: Canine coronavirus-associated puppy mortality without evidence of concurrent canine parvovirus infection. J. Vet. Diagn. Invest. 2005, 17, 610-614.
16. *Herrewegh A. A., Smeenk I., Horzinek M. C., Rottier P. J., DeGroot R. J.*: Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. J. Virol. 1998, 72, 4508-4514.
17. ICTV – International Committee on Taxonomy of Viruses. Virus Taxonomy: 2012 release. http://www.ictvonline.org/virusTaxonomy.asp, accessed 09.03. 2014.
18. *Keenan K. P., Jervis H. R., Marchwicki R. H., Binn L. N.*: Intestinal infection of neonatal dogs with canine coronavirus 1-71: studies by virologic, histologic, histochemical, and immunofluorescent techniques. Am. J. Vet. Res. 1976, 37, 247-256.
19. *Mitchell J. A., Brooks H. W., Szladovits B., Erles K., Gibbons R., Shields S., Brownlie J.*: Tropism and pathological findings associated with canine respiratory coronavirus (CRCoV). Vet. Microbiol. 2013, 162, 582-594.
20. *Möstl K., Buxbaum A., Odörfer G.*: Verbreitung und Bedeutung von Coronavirus-Infektionen in heimischen Hundepopulationen. Wien. Tierärztl. Monat. – Vet. Med. Austria 1994, 81, 355-361.
21. *Ntafis V., Mari V., Decaro N., Papanastassopoulou M., Pardali D., Rallis T. S., Kanellos T., Buonavoglia C., Xylouri E.*: Canine coronavirus, Greece. Molecular analysis and genetic diversity characterization. Infection, Genetics and Evolution 2013, 16, 129-136.
22. *Pratelli A., Decaro N., Tinelli A., Martella V., Elia G., Tempesta M., Cirone F., Buonavoglia C.*: Two genotypes of canine coronavirus simultaneously detected in fecal samples of dogs with diarrhea. J. Clin. Microbiol. 2004, 42, 1797-1799.
23. *Pratelli A., Elia G., Martella V., Palmieri A., Cirone F., Tinelli A., Corrente M., Buonavoglia C.*: Prevalence of canine coronavirus antibodies by an enzyme--linked immunosorbent assay in dogs in the south of Italy. J. Virol. Methods 2002, 102, 67-71.
24. *Pratelli A., Elia G., Martella V., Tinelli A., Decaro N., Marsilio F., Buonavoglia D., Tempesta M., Buonavoglia C.*: M gene evolution of canine coronavirus in naturally infected dogs. Vet. Rec. 2002, 151, 758-761.
25. *Pratelli A., Martella V., Decaro N., Tinelli A., Camero M., Cirone F., Elia G., Cavalli A., Corrente M., Greco G., Buonavoglia D., Gentile M., Tempesta M., Buonavoglia C.*: Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy. J. Virol. Methods 2003, 110, 9-17.
26. *Pratelli A., Martella V., Elia G., Decaro N., Aliberti A., Buonavoglia D., Tempesta M., Buonavoglia C.*: Variation of the sequence in the gene encoding for transmembrane protein M of canine coronavirus (CCV). Mol. Cell. Probes 2001, 15, 229-233.
27. *Pratelli A., Tempesta M., Roperto F. P., Sagazio P., Carmichael L. E., Buonavoglia C.*: Fatal coronavirus infection in puppies following canine parvovirus 2b infection. J. Vet. Diagn. Invest. 1999, 11, 550-553.
28. *Pratelli A., Tinelli A., Decaro N., Camero M., Elia G., Gentile A., Buonavoglia C.*: PCR assay for the detection and the identification of atypical canine coronavirus in dogs. J. Virol. Methods 2002, 106, 209-213.
29. *Priestnall S. L., Brownlie J., Dubovi E. J., Erles K.*: Serological prevalence of canine respiratory coronavirus. Vet. Microbiol. 2006, 115, 43-53.
30. *Priestnall S. L., Pratelli A., Brownlie J., Erles K.*: Serological prevalence of canine respiratory coronavirus in southern Italy and epidemiological relationship with canine enteric coronavirus. J. Vet. Diagn. Invest. 2007, 19, 176-180.
31. *Rennhofer M., Benetka V., Sommerfeld-Stur I., Möstl K.*: Epidemiological investigations on coronavirus infections in dogs and cats in an animal shelter. Wien. Tierärztl. Monat. – Vet. Med. Austria 2005, 92, 21-27.
32. *Spiss S., Benetka V., Künzel F., Sommerfeld-Stur I., Walk K., Latif M., Möstl K.*: Enteric and respiratory coronavirus infections in Austrian dogs: serological and virological investigations of prevalence and clinical importance in respiratory and enteric disease. Wien. Tierärztl. Monat. – Vet. Med. Austria 2012, 99, 67-81.
33. *Stavisky J., Pinchbeck G., Gaskell R. M., Dawson S., German A. J., Radford A. D.*: Cross sectional and longitudinal surveys of canine enteric coronavirus infection in kennelled dogs: a molecular marker for biosecurity. Infection, Genetics and Evolution 2012, 12, 1419-1426.

Corresponding author: Ao. Univ.-Prof. Dr. med. vet. Karin Möstl, Institute of Virology, Department for Pathobiology, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria; e-mail: Karin.Moestl@vetmeduni.ac.at, karinmoestl@gmail.com