

Canine Respiratory Coronavirus: An Emerging Pathogen in the Canine Infectious Respiratory Disease Complex

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Respiratory disease in dogs is generally of greatest importance in establishments in which dogs are housed in groups, such as shelters, boarding kennels, and veterinary hospitals. Disease outbreaks involving only one species of infectious agent are possible, as seen in distemper outbreaks in susceptible populations [1]. Most commonly, however, infectious respiratory disease in dogs has a multifactorial etiology and is best described as canine infectious respiratory disease (CIRD) complex (also known as “kennel cough”).

Viruses detected in dogs with CIRD include canine parainfluenza virus (CPIV) [2], canine adenovirus (CAV) type 2 [3] and canine herpesvirus [4]. Canine influenza virus, which recently has been detected in some parts of the United States, is likely to become part of the disease complex because it often causes mild respiratory disease characterized by nasal discharge and persistent cough [5].

Bacteria are important in CIRD as primary pathogens and as a cause of secondary infections. *Bordetella bronchiseptica* is the bacterium most frequently associated with CIRD [6], but mycoplasmas, particularly *Mycoplasma cynos*, have also been linked to the disease [6,7]. *Streptococcus equi* subsp. *zooeptidemicus* has been isolated from severe cases of respiratory disease that were frequently fatal [8]. Vaccines have been developed for protection against several canine respiratory pathogens. Combination vaccines routinely contain canine distemper virus and CAV-1 or CAV-2. CAV vaccines confer cross-protection against type 1 (the cause of canine infectious hepatitis) and type 2 (associated with respiratory disease). CPIV is also included in several multivalent vaccines, or it is available in combination with *B bronchiseptica* as a “kennel cough vaccine.”

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These are generally formulated for intranasal administration to provide a fast mucosal immune response. Despite the widespread use of vaccines, CIRDC is an ongoing problem in many kennels. Possible causes are a lack of protection because of antigenic variants, the presence of known infectious agents for which vaccines have not yet been developed (eg, mycoplasmas), and the presence of novel infectious agents.

ORIGINS OF CANINE RESPIRATORY CORONAVIRUS

Canine respiratory coronavirus (CRCoV) was first detected in 2003 in dogs housed at a UK rehoming center [9]. The center had a high turnover of dogs and was reporting problems with enzootic respiratory disease despite regular vaccination. An investigation into pathogens associated with CIRDC in this population led to the detection of a coronavirus in tracheal and lung samples by reverse transcriptase polymerase chain reaction (RT-PCR).

Coronaviruses are large enveloped viruses containing a positive-sense single-stranded RNA genome. The structural proteins located in the viral envelope include the spike protein (S), the membrane protein (M), and the small membrane protein (E). Initial sequence analysis of CRCoV showed a high similarity to bovine coronavirus (BCoV) and human coronavirus OC43 (96% amino acid identity with BCoV in the variable spike protein).

Coronaviruses had been described before in dogs with gastroenteritis [10]; however, it was shown that CRCoV was distinct from the previously known canine coronavirus (CCoV). The virus showed only 69% nucleotide identity in the highly conserved polymerase region and only 21% amino acid sequence identity in the spike protein, indicating that CRCoV was a novel coronavirus of dogs.

Members of the family Coronaviridae are separated into groups according to their genetic similarities [11]. Most members of group 2 of coronaviruses contain an additional gene coding for a surface hemagglutinin-esterase protein. This gene was found to be present in CRCoV, confirming its place in group 2 together with its closest relative, BCoV (Fig. 1). CCoV, in contrast is a member of group 1, which includes feline coronavirus and porcine transmissible gastroenteritis among others.

Currently, the oldest samples that tested positive for CRCoV are canine lung samples collected in Canada in 1996 [12]. One of the reasons precluding earlier discovery of CRCoV may be its poor growth in cell culture and the requirement for specific host cells. The close genetic relation to BCoV throughout the CRCoV genome indicates that the virus was probably transmitted to dogs from cattle [13]. Interestingly, it has recently been shown that human coronavirus OC43 also may have emerged after viral transmission from cattle to people [14].

EPIDEMIOLOGY

Coronaviruses are a cause of respiratory disease in many species, including human beings, poultry, and cattle [15–20]. The presence of CRCoV in dogs was first described in a large study of dogs with CIRDC [9]. In this investigation,

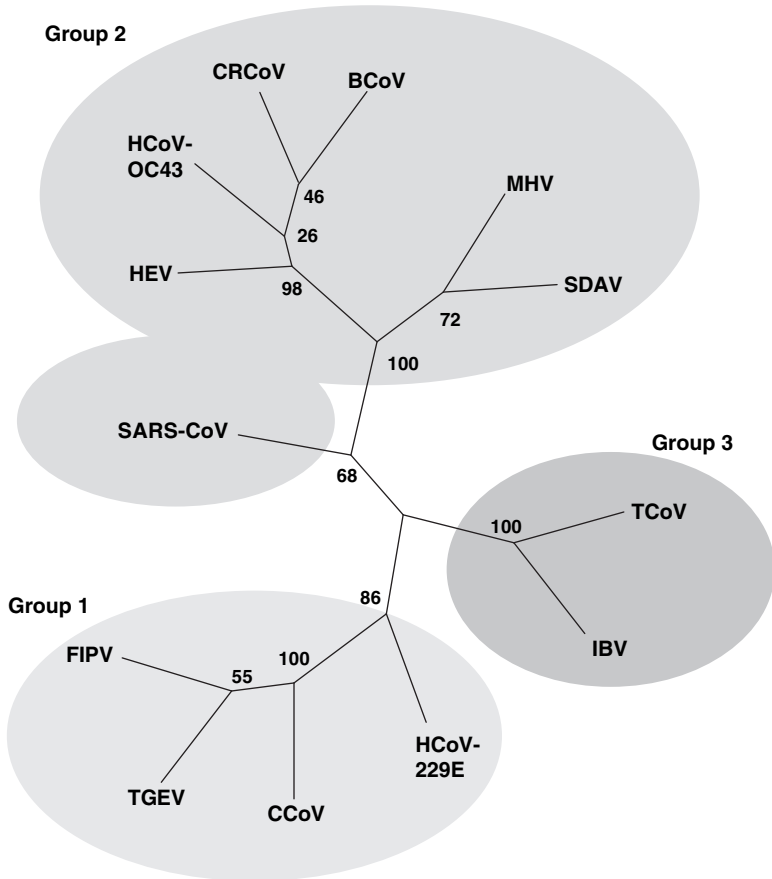


Fig. 1. Phylogenetic tree based on the partial polymerase gene sequence of coronaviruses. Gray shaded areas show the separation into groups 1 to 3. CRCoV is situated in group 2 with the most closely related species, BCoV, human coronavirus strain OC43 (HCoV-OC43), and porcine hemagglutinating encephalomyelitis virus (HEV), in addition to murine hepatitis virus (MHV) and rat sialodacryoadenitis virus (SDAV). Group 1 contains enteric CCoV, porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV), and human coronavirus strain 229E (HCoV-229E). Group 3 contains the avian coronaviruses infectious bronchitis virus (IBV) and turkey coronavirus (TCoV). Severe acute respiratory syndrome (SARS) coronavirus is currently classified as part of group 2 but may be reclassified as a new group 4 with related bat coronaviruses.

performed at a shelter, clinical signs were graded by veterinary clinicians into (1) no signs of respiratory disease; (2) mild cough; (3) mild cough and nasal discharge; (4) cough, nasal discharge, and inappetence; and (5) severe respiratory disease with evidence of bronchopneumonia. Because of a small number of samples in grade 4, grades 3 and 4 were merged and referred to as “moderate respiratory disease.” CRCoV was most frequently detected in the trachea

of dogs with mild clinical signs (grade 2). It was less frequently recovered from dogs with moderate or severe clinical signs or from dogs without clinical signs at the time of sampling. CRCoV was also detected in the lung, albeit less frequently. Table 1 summarizes the detection of CRCoV in clinical samples from dogs.

After 3 weeks of stay at a shelter, almost 100% of dogs tested positive for antibodies to CRCoV compared with 30% on the day of entry, indicating that the virus was highly prevalent in the population and was easily transmitted. It was also found that the presence of antibodies to CRCoV on the day of entry led to a significantly reduced risk for contracting CIRDC, supporting the hypothesis that CRCoV played a role in the etiology of the disease [9].

After this initial investigation, CRCoV was also detected in two UK training kennels for working dogs [21]. Serum samples had been collected during two outbreaks of respiratory disease at one of the kennels and 4 weeks after. Almost all dogs housed at the kennel showed seroconversion to CRCoV after the outbreaks. Moreover, CRCoV was detected by PCR in two oropharyngeal swabs taken from dogs with clinical respiratory disease. Not all dogs that developed antibodies to CRCoV also showed signs of CIRDC; nevertheless, this was the second study associating CRCoV with respiratory disease in dogs.

Table 1
Detection of canine respiratory coronavirus in clinical samples from dogs

Sample type	Clinical signs or histopathologic diagnosis	No. CRCoV-positive samples out of total no. samples (%)	Reference
Trachea	None	11 of 42 (26.1)	[9]
	Mild respiratory disease ^a	10 of 18 (55.6)	[9]
	Moderate respiratory disease	9 of 46 (19.6)	[9]
	Severe respiratory disease	2 of 13 (15.4)	[9]
Lung	None	8 of 42 (19)	[9]
	Mild respiratory disease	4 of 18 (22.2)	[9]
	Moderate respiratory disease	8 of 46 (17.4)	[9]
	Severe respiratory disease	0 of 13	[9]
Lung	Severe gastroenteritis ^b	1 of 109 (0.92)	[26]
Lung	Bronchitis/bronchiolitis	2 of 126 (1.6)	[12]
Oropharyngeal swab	Mild respiratory disease	2 of 64 (3.1)	[21]
Oropharyngeal swab	None	1 of 64 (1.6)	[21]
Oral swab	Cough	1 of 10 (10)	[23]
Oral swab	None	1 of 10 (10)	[23]
Nasal swab	Cough and nasal discharge	1 of 59 (1.7)	[22]
Rectal swab	Gastroenteritis ^c	1 of 65 (1.5)	[22]

^aCriteria for grading into mild, moderate, and severe respiratory disease are explained in the section on epidemiology.

^bEvidence of bronchopneumonia at postmortem examination, canine parvovirus, and CCoV also detected.

^cCCoV and CPIV also detected.

Two further studies identified CRCoV in nasal and oral swabs from dogs in Japan that had respiratory disease [22,23]. An analysis of 126 archival tissue blocks of cases of respiratory disease identified two CRCoV-positive samples by immunohistochemistry. Both were from dogs with bronchitis and bronchiolitis, and CRCoV antigen was found to be present in respiratory columnar epithelial cells. One of those dogs was also positive for canine distemper virus [12]. The detection rate of CRCoV in the archival study may seem quite low; however, the tissue samples were mostly derived from dogs with severe respiratory disease with a fatal outcome, whereas CRCoV has mostly been associated with mild respiratory disease so far.

Serologic studies to determine the prevalence of antibodies to CRCoV have been performed to date for the United Kingdom, Republic of Ireland, Italy, United States, Canada, and Japan. The highest seroprevalence was detected in Canada (59.1% of sera tested were found to be positive) and the United States (54.7% of sera tested were found to be positive). Samples from the United States had been collected from 33 states, and positive samples were identified in 29 [24]. In those states that allowed a meaningful interpretation of seroprevalence (more than 10 samples), the prevalence ranged from 31.3% (Maine) to 87.5% (Kentucky). The prevalence in the United Kingdom and the Republic of Ireland was lower, with 36% and 30.3%, respectively [24]. Two studies performed in Italy showed seroprevalence ranging from 20% to 32.5% [25,26]. The lowest seroprevalence was detected in Japan, with 17.8% [23]. Further data are not yet available, but it is likely that CRCoV is present throughout the United States and in other European countries.

Although CRCoV was detected throughout the year in a kennel with enzootic CIRDC, another study reported a seasonal occurrence of the virus in the winter months. No seroconversions to CRCoV and few cases of respiratory disease were recorded in the summer months [21]. A similar seasonality has been reported for human coronaviruses involved in the common cold [27].

CRCoV infections can occur in dogs of all ages. Dogs younger than 1 year of age were significantly more likely to be seronegative than older dogs, however [24,25]. This is in contrast to the prevalence of enteric CCoV, which is frequently found in dogs younger than 1 year of age [10]. This may reflect different patterns of transmission of the two viruses. The seroprevalence of CRCoV was increasing after the age of 1 year for all studies and then reached a plateau between the ages of 2 and approximately 8 years. This is probably a consequence of the greater probability of exposure to the virus with increasing contact with other dogs. It is not certain how long CRCoV antibody levels in dogs remain stable after infection. One study showed a twofold decrease in antibody titers in 6 of 14 dogs tested and a fourfold decrease in 4 dogs in less than 1 year [21]. Because these were naturally occurring infections, it is not known if the antibody response measured reflected primary or repeated infections. The viral dose encountered by dogs would also influence the level and duration of the antibody response.

The rapid spread of CRCoV through kennel populations indicates that the virus is highly contagious. This, in conjunction with the predominant detection of CRCoV in respiratory samples, suggests that it is mostly spread by means of respiratory secretions. CRCoV probably enters the respiratory tract by inhalation of droplets or contact with secretions and contaminated surfaces.

PATHOGENESIS AND CLINICAL SIGNS

It is not possible to discuss the pathogenesis and clinical signs associated with CRCoV without considering the CIRDC complex as a whole. CRCoV has been detected in several studies in dogs with respiratory disease. In most of these cases, however, other respiratory pathogens were also present. In two detailed studies into the causes of CIRDC in which evidence of CRCoV was reported, the dogs presented with the typical signs of a dry cough and nasal discharge [21,28]. Concurrent infections were most frequently caused by CPIV and *B bronchiseptica*.

CRCoV has also been detected in dogs that have nonrespiratory disease. It was detected in the lung, spleen, mesenteric lymph nodes, and intestines of a dog that had died from hemorrhagic gastroenteritis [26]. The dog also tested positive for canine parvovirus type 2 and CCoV. Similarly, CRCoV was detected in a rectal swab from a dog with vomiting and diarrhea, which was also positive for CCoV and CPIV [22]. In both cases, the concurrent infections with canine parvovirus or CCoV are likely to have been the cause of the clinical signs. Studies of the tissue distribution of CRCoV in 10 naturally infected dogs showed that CRCoV was most frequently detected in the nasal cavity, nasal tonsil, and trachea and less frequently in the lung, bronchial lymph nodes, and palatine tonsil. It was also detected in samples from the spleen, mesenteric lymph nodes, and colon but not in the enteric content (K. Erles, unpublished data, 2004). The tissue tropism of CRCoV therefore seems not to be exclusively respiratory, and fecal-oral transmission of CRCoV may be possible.

CRCoV may show a dual tropism, similar to BCoV, but the ability of the virus to replicate in the epithelium of the gastrointestinal tract and the clinical consequences need further investigation.

Experimental studies using CRCoV have not been reported to date. A study using BCoV showed that dogs became infected and transmitted the virus to contact dogs. BCoV was detected in rectal and oral swabs, and the dogs developed neutralizing antibodies to the virus [29]. The dogs did not develop fever or any clinical signs of respiratory or gastrointestinal disease. Despite their high similarity, BCoV may be less pathogenic in dogs compared with CRCoV. Furthermore, the etiology of CIRDC has been shown to involve multiple pathogens. Viral infections can aid the entry of other pathogens by facilitating their attachment or by inhibition of the mucociliary clearance. Many pathogens known to be involved in the CIRDC complex have also been found in dogs without clinical signs, including CPIV and *B bronchiseptica* [28,30]. When assessing the pathogenesis of complex diseases, it is important to consider the possible interaction of pathogens during coinfections and contributing factors, such as stress.

DIAGNOSIS

Because of the involvement of multiple pathogens in the etiology of CIRDC, it is not possible to diagnose CRCoV solely by clinical signs. The most suitable test to diagnose CRCoV in respiratory samples is nested RT-PCR based on the spike glycoprotein gene [9] or the hemagglutinin-esterase gene [22]. This test has a high sensitivity, which is particularly useful when analyzing samples with a potentially low number of cells, such as oropharyngeal or nasal swabs. Both PCR methods are specific for CRCoV and do not detect enteric CCoV. Because the virus was found most frequently in the nasal cavity, nasal swabs are suitable diagnostic samples. CRCoV has also been detected in oral swabs, and, furthermore, nasal or tracheal washes are likely to yield CRCoV during an active infection. If postmortem samples are available, nasal cavity, nasal tonsil, trachea, and lung samples should be collected for analysis.

Isolation of CRCoV in cell culture has been achieved; however, it is not recommended to use virus isolation alone to diagnose CRCoV. To date, isolation of CRCoV has only succeeded on the human rectal tumor cell line HRT-18 and its clone HRT-18G [13]. Even on HRT-18 cells, the isolation of CRCoV from RT-PCR-positive samples is often unsuccessful [22,23,26]. The only isolate of CRCoV that has so far been studied in detail did not produce a cytopathic effect on HRT-18 cells, and infection had to be confirmed by using immunofluorescence or PCR. Supernatants from infected cell cultures were found to agglutinate chicken erythrocytes at 4°C [13]. Hemagglutination assays may aid in detection of CRCoV-infected cell cultures if isolation is attempted.

CRCoV has also been detected by immunohistochemistry on formalin-fixed tissues using an antibody directed against BCoV [12]. The sensitivity of immunohistochemistry in comparison to PCR has not been evaluated; however, this method is useful for testing archival respiratory samples.

Serology is a valuable tool for the detection of CRCoV infections if paired serum samples are collected during an outbreak of respiratory disease and at least 2 to 3 weeks afterward. The high similarity of CRCoV and BCoV allows the use of BCoV antigens to test canine sera by ELISA [9,26]. Similarly, BCoV has been used instead of CRCoV in serum neutralization tests [23]. A hemagglutination inhibition test based on BCoV has also been evaluated but was assessed as having poor sensitivity and specificity compared with an ELISA based on BCoV [26].

An ELISA assay using CRCoV antigen was found to have slightly higher sensitivity and specificity compared with an assay based on BCoV; however, overall, the agreement between the two ELISA tests was high [24]. Antibodies to CRCoV have also been detected by using an immunofluorescence assay on CRCoV-infected HRT-18 cells [24].

Specific tests for CRCoV are becoming increasingly available; however, most assays offered for the detection of coronaviruses in dogs are specific for enteric CCoV. Antibodies to CRCoV do not cross-react with enteric CCoV. It is important to use an assay capable of detecting antibodies to CRCoV or related group 2 coronaviruses, such as BCoV. Consequently, the requirement

for CRCoV detection should be discussed with the diagnostic laboratory before submitting samples for RT-PCR, virus isolation, or serology.

TREATMENT

There is no specific treatment for infections caused by CRCoV. As for other causes of CIRD, patient care should focus on the prevention and treatment of bacterial infections. Although many pathogens involved in CIRD are reported to be associated with mild clinical disease, it is important to bear in mind that mixed infections can potentially be much more severe. Patients should be monitored, because the condition may rapidly worsen. Severe cases of CIRD with sudden death have been reported after infections with *Streptococcus equi* subsp. *zooepidemicus* [8].

PREVENTION

To date, no vaccines against CRCoV are available. Vaccines against CCoV are unlikely to protect against infection with CRCoV because of a low similarity in the spike proteins that are the major immunogenic proteins of coronaviruses. Vaccines against other respiratory pathogens may not prevent CIRD, particularly in large populations because of the presence of other infectious agents. Nevertheless, they have the potential to reduce the number of circulating pathogens if given to all dogs on entry. Vaccines against canine distemper virus and CAV are widely used, and this may account for the inability to identify either virus in a population with enzootic CIRD [28].

Although no specific tests have been performed to determine the stability of CRCoV in the environment, other coronaviruses have been reported to remain infectious in respiratory secretions for more than 7 days [31]. Thorough cleaning and disinfection of kennels after outbreaks of respiratory disease are therefore required. Coronaviruses are inactivated by disinfectants commonly used for surface disinfection in kennels and veterinary practices. The role of fecal shedding and the potential transmission of CRCoV among dogs sharing common facilities, such as outdoor runs, have yet to be resolved. Other generally recommended measures, such as washing one's hands after handling animals with respiratory disease should also help to reduce the spread of the virus. CRCoV has been detected in dogs up to 4 weeks after entry into a kennel. Because the time of infection in those naturally occurring cases is not known, it is unclear how long CRCoV is being shed. After experimental infection of dogs with BCoV, the virus was detected in a rectal swab after 11 days [29]. Quarantine of newly arriving dogs, if feasible in training kennels or shelters, should therefore last for at least 2 weeks.

PRESENCE OF GROUP 1 CANINE CORONAVIRUS IN THE RESPIRATORY TRACT

Group 1 CCoVs, referred to in this article as CCoVs, have previously been associated with mild gastroenteritis. According to their similarity to feline coronaviruses, they are divided into type I (related to feline coronavirus type I) or type II (related to feline coronavirus type II) [32]. Although CCoV has been

isolated from the lung after experimental infections [33], it was generally considered to be restricted to the gastrointestinal tract during naturally occurring infection. Recently, an outbreak of a systemic fatal disease was described from which a type II CCoV was isolated [34]. Although the dogs presented with vomiting, diarrhea, and neurologic signs, postmortem examination also revealed bronchopneumonia. CCoV was detected in internal organs, including the lung, kidney, and brain. Sequence analysis of the CCoV isolate identified a mutation in open reading frame 3b, leading to a truncated nonstructural protein. It is not clear if this mutation is responsible for the extended tropism of this isolate [35]. Further studies are required to determine the presence of type II CCoVs in cases of severe systemic disease and in cases of respiratory disease.

SUMMARY

CRCoV is a novel coronavirus of dogs distinct from CCoV. It is present in North America, Europe, and Japan. CRCoV is frequently detected in dogs with clinical respiratory signs and may contribute to the CIRDC complex. Increased awareness of the existence of CRCoV and the development of routinely available diagnostic tests should enhance our knowledge of the presence of CRCoV in canine populations with and without respiratory disease. It is recommended to use PCR methods or serology on paired serum samples to diagnose CRCoV infections, because the sensitivity of virus isolation is low. Identification of causative agents during outbreaks of respiratory disease in canine populations ought to be performed more frequently. This would help to determine the importance of individual viruses and bacteria, not only in the investigated population but in the CIRDC complex as a whole. The etiology of CIRDC is multifactorial and is likely to change continuously, because some pathogens are controlled by vaccination, although other infectious agents emerge to take their place.

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