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Short Communication

The seroprevalence of canine respiratory coronavirus and canine influenza virus in dogs in New Zealand

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Abstract

AIM: To determine whether canine respiratory coronavirus (CRCoV) and canine influenza virus (CIV) are present in dogs in New Zealand.

METHODS: Serum samples from 251 dogs of varying age, breed and clinical histories were tested for the presence of antibodies to CRCoV and CIV, using indirect fluorescent antibody (IFA) analysis. The population sampled represented a wide geographic area but principally encompassed the central and lower North Island of New Zealand.

RESULTS: Seventy-three of the 251 samples (29%) were seropositive for CRCoV. Dogs <2 years old were less likely to be seropositive for CRCoV than older dogs. None was seropositive for CIV.

CONCLUSIONS: This study revealed the presence of antibodies to CRCoV in dogs in New Zealand. Young dogs are less likely to be seropositive than older dogs, probably due to increased opportunity for exposure to CRCoV over time. Serum antibodies to CIV were not detected in any of the dogs sampled, suggesting that this virus is unlikely to be present in dogs in New Zealand.

CLINICAL RELEVENCE: Canine respiratory coronavirus is present in New Zealand. Although the role of this virus in canine infectious tracheobronchitis has not been fully elucidated, evidence suggests that it may have a causal role in this disease. Veterinarians should consider CRCoV as a differential diagnosis in cases of respiratory disease in dogs in New Zealand. While CIV appears not to be currently present in New Zealand, veterinarians should consider infection with this virus as a differential diagnosis in dogs presenting with respiratory signs.

KEY WORDS: Canine respiratory coronavirus, CRCoV, canine influenza virus, CIV, New Zealand, serology, indirect fluorescent antibody, IFA

Introduction

Coronaviruses have been associated with respiratory disease in a number of species, including dogs and humans (Erles et al. 2003; Ellis et al. 2005; Priestnall et al. 2006). One study identified a novel canine respiratory coronavirus (CRCoV) in the respiratory tract of dogs in a large re-homing kennel in the United Kingdom (UK) where canine infectious tracheobronchitis was endemic and not controlled by the use of vaccines currently available against respiratory pathogens (Erles et al. 2003). This finding suggested a role for CRCoV and possibly other unidentified pathogens in the aetiology of canine infectious tracheobronchitis (Priestnall et al. 2006). CRCoV has been implicated as an important and prevalent infectious agent in many cases of respiratory disease in dogs in humane shelters in the UK (Erles et al. 2003, 2004; Erles and Brownlie 2005; Priestnall et al. 2006). A retrospective study in Canada provided further serological evidence that CRCoV may be causally associated with airway disease in dogs (Ellis et al. 2005), and studies in Japan and southern Italy suggested that CRCoV was prevalent amongst dogs there (Kaneshima et al. 2006; Yachi and Mochizuki 2006; Priestnall et al. 2007). CRCoV, a Group 2 coronavirus, is quite distinct from the canine enteric coronavirus (CCoV), a Group 1 coronavirus associated with gastrointestinal disease in puppies. Due to the dissimilarity in the major immunogenic viral spike protein between CRCoV and CCoV, neither infection with CCoV nor the use of vaccines currently marketed against CCoV is expected to illicit a neutralising or protective response to CRCoV infection (Erles and Brownlie 2008).

Canine influenza virus is a newly emerging and highly contagious respiratory tract pathogen of dogs caused by Influenza A subtype H3N8 (Crawford et al. 2005; Smith and Daly 2005; Newton et al. 2007). This virus was first isolated from the lungs of racing Greyhounds that died of haemorrhagic pneumonia during outbreaks of respiratory disease at racetracks in Florida, United States of America (USA), during 2003 and 2004 (Crawford et al. 2005, 2007). This virus was first isolated from the lungs of racing Greyhounds that died of haemorrhagic pneumonia during outbreaks of respiratory disease at racetracks in Florida, United States of America (USA), during 2003 and 2004 (Crawford et al. 2005, 2007). Seroposiemical studies indicated efficient and widespread horizontal transmission of CIV between pet dogs in the USA. As a naïve population, almost all dogs, regardless of breed or age, appeared to be susceptible due to lack of immunity. About 80% of dogs infected with CIV had clinical disease while 20% had subclinical infections (Crawford et al. 2007). Thus far, two clinical syndromes have been described, viz a mild form involving the upper respiratory tract and a more severe form in the lower respiratory tract, complicated by secondary infections. It is possible that some dogs with a dry cough associated with upper respiratory tract infection may have been infected with CIV (Crawford et al. 2007).

CCoV  Canine enteric coronavirus
CIV  Canine influenza virus
CRCoV  Canine respiratory coronavirus
EIV  Equine influenza virus(es)
HRT18G  Human rectal tumour
IFA  Indirect fluorescent antibody

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Canine influenza virus was determined to be very closely related to contemporary equine influenza viruses (EIV) (Crawford et al. 2005, 2007). Those authors concluded that the CIV outbreaks first reported in Greyhounds in March 2003 represented an unprecedented interspecies transfer of a complete EIV to dogs and consequent emergence of a new influenza virus associated with acute respiratory disease. A limited but fatal outbreak of respiratory disease attributable to H3N8 EIV infection in Quarryhounds from Britain was retrospectively confirmed in the autumn of 2002 (Smith and Daly 2005), and a further retrospective diagnosis of EIV infection was made among Foxhounds in Britain (Newton et al. 2007). Equine influenza occurs widely throughout the world, and until the recent outbreak in Australia in 2007, Australia and New Zealand were the only two countries with significant populations of horses that had been free of the virus (Daly and Mumford 2001).

To date, it has not been established whether either CRCoV or CIV are present in New Zealand. In light of the recent outbreak of equine influenza in Australia and the frequent movement of horses and dogs between Australia and New Zealand, we set out to determine whether CIV is currently present in New Zealand. In every country studied to date, there is a high prevalence of serum antibodies to CRCoV in samples collected from dogs. It was therefore our hypothesis that we would also find dogs seropositive to this virus in New Zealand.

Materials and methods

Blood samples
Blood samples obtained from 251 dogs that had been submitted by veterinary practitioners to a private veterinary diagnostic laboratory (New Zealand Veterinary Pathology Ltd, Palmerston North, NZ) were used. The samples obtained were from a range of breeds of dogs of various ages and with varying clinical histories. The blood samples were submitted as part of diagnostic work-ups for a range of clinical and non-clinical conditions, and histories of varying detail accompanied each sample. The population sampled represented a wide geographic area encompassing the central and lower North Island of New Zealand. Serum was harvested and frozen at −20°C until used.

IFA procedure for CRCoV and CIV
Indirect fluorescent antibody procedures were conducted in a laboratory located in the USA. Monolayers of canine kidney cells of USA origin and human rectal tumour (HRT18G; American Type Culture Collection Cell Line, Manassas VA, USA) planted in 96-well tissue-culture plates were cultured to 30–70% confluency in Dulbecco’s modified eagle’s medium (Sigma-Aldrich Fine Chemicals Biosciences, St Louis MO, USA) supplemented with fetal bovine serum (Sigma-Aldrich Fine Chemicals Biosciences). Monolayers were inoculated with 25 tissue-culture infectious doses/well of CRCoV or CIV virus isolates originating from the USA. Infected and non-infected monolayers were incubated in a humidified incubator at 37°C for 2–5 days, and then fixed using 80% acetone (in water). Each canine serum sample was diluted 1:40 in phosphate buffered saline supplemented with 1% bovine serum albumin (w/v) and 0.09% sodium azide (w/v), and added at 50 μl/well. The conjugate was incubated at room temperature for 40–60 minutes, followed by washing the plates twice in water. Endpoint CRCoV and CIV IFA titres, observed using immunofluorescence microscopy inverted microscope (Olympus IX71/IX51; Olympus, Southend-on-Sea, Essex, UK) fitted with a xenon 75-W lamp and fluorescein isothiocyanate filters, were the inverse of the last dilution of serum exhibiting definite CRCoV or CIV fluorescence. In instances where no virus-specific fluorescence at the 1:40 dilution was observed, dogs were considered seronegative or non-exposed to the virus. Each 96-well plate included both positive and negative control antibody. Bovine coronavirus direct fluorescent antibody (American BioResearch Inc, Pullman WA, USA) and a non-commercial source of CIV-positive sera were used as positive controls for CRCoV and CIV, respectively. Serum from dogs known to be CRCoV and CIV antibody-negative by IFA assay was used as negative control antibody.

Statistical analysis
Dogs were grouped in the following age groups: 0–2 years, 3–6 years, 7–10 years, and 11–18 years. The overall prevalence of dogs seropositive for both CRCoV and CIV was determined within each age group. A χ² test was used to determine whether there were any effects of age on the prevalence of dogs seropositive for CRCoV. In addition, a χ² test was used to determine whether there was an effect of age group on the proportion of samples that were seropositive within each of the antibody titre levels of CRCoV (<1:40, 1:160, 1:320, 1:640, and >1:1,280). The χ² test was used to determine whether there was any difference in the prevalence of dogs seropositive for CRCoV between those with and those without a history of clinical signs consistent with respiratory disease. Minitab v15 (Minitab Inc, State College PA, USA) was used for statistical calculations.

Results
Of 251 sera, 73 (29%) were found to be seropositive for CRCoV. None was seropositive for CIV. The prevalence of samples with antibodies to CRCoV differed between dogs when grouped according to age (p=0.045), due mainly to fewer than expected antibody-positive dogs within the 0–2-year-old age group (Figure 1). However, there was no effect of age group on the proportion of seropositive samples within each of the CRCoV antibody titre levels (p=0.109).

Of the 251 samples tested, a history was submitted with the sample in 210 (84%) cases. Of those 210 samples, 63 (30%) dogs were seropositive to CRCoV. Of this subset, 2/9 (22%) dogs were seropositive and had a history of respiratory signs and 61/201
Various studies have shown that infection with CRCoV can occur in dogs of all ages but dogs younger than 1 year of age are more likely than older dogs to be seronegative (Priestnall et al. 2006, 2007; Erles and Brownlie 2008). The seroprevalence of CRCoV has been found to increase in dogs older than 1 year of age, reaching a plateau between the ages of 2 and 8 years (Erles and Brownlie 2008). This pattern is thought to be a consequence of increasing likelihood of exposure to CRCoV, with increasing contact with infected dogs over time (Erles and Brownlie 2008), although the persistence of CRCoV antibody titres post-infection has not been determined. Our findings are consistent with this pattern as there was a lower prevalence of CRCoV-seropositive dogs in the 0–2-year-old age group than in the older age groups, and the prevalence within the older age groups was comparable with earlier reports in the literature (Figure 1).

As well as considering the prevalence of dogs seropositive to CR-CoV, we also wished to determine whether there was an effect of age group on the proportion of seropositive samples within different titre levels of CRCoV antibody. Low titres in older dogs may reflect antibodies acquired during past exposure rather than recent infection, and correspondingly high titres in older dogs may be suggestive of frequent re-exposure to the virus. However, no effect of age group on the magnitude of the titres was found.

Canine infectious tracheobronchitis, also known as kennel cough, is a multi-aetiological condition which can be caused by viruses and bacteria, including mycoplasmas (Erles et al. 2003; Ellis et al. 2005; Ford 2006; Priestnall et al. 2007; Zeugwetter et al. 2007). In a re-homing kennel, Erles et al. (2003) found that endemic canine infectious tracheobronchitis could not be controlled by routine vaccination against some of the known pathogens, namely canine parainfluenza virus, canine adenovirus type 2, and Bordetella bronchiseptica. Those authors found that dogs that had no antibodies to CRCoV on entry into the kennel had a significantly increased probability of developing respiratory disease. This finding suggests that CRCoV may be involved in the pathogenesis of canine infectious tracheobronchitis.

We were interested in determining whether there was an increased seroprevalence to CRCoV in dogs that had a history consistent with respiratory disease, but this was not seen. There are many potential reasons for this. Firstly, antibody persistence in dogs infected with CRCoV is unknown. Dogs seropositive to CRCoV but lacking a clinical history consistent with respiratory disease may reflect past exposure to CRCoV. Secondly, an apparent lack of association between seroprevalence for CRCoV and clinical disease could be explained by sampling acutely infected dogs before they have had a chance to seroconvert. Access to paired acute and convalescent serum samples was not available in this retrospective study; such samples would be required in order to further understand this association more clearly. Thirdly, the lack of association could have been due to the fact that dogs with a history of respiratory signs may have had non-respiratory disease, e.g. heart disease, or may have had primary respiratory disease of a non-infectious nature, e.g. neoplasia, or infectious respiratory disease due to another cause. The retrospective nature of this study made it impossible to separate out these possibilities. Additionally, it is important to consider the close relationship between CRCoV and bovine coronavirus (Erles et al. 2003). Dogs experimentally infected with bovine coronavirus did not suffer from significant disease (Kaneshima et al. 2007). As such, a fourth possibility to consider is that, given this was a serological study, it was not pos-

![Figure 1. Distribution of serum samples with positive (c) or negative (a) antibody status for canine respiratory coronavirus (CRCoV) from 251 dogs in New Zealand differing in age. The prevalence of CRCoV within each age group is shown above the bars.](image-url)
sible to differentiate between infection with CRCoV or bovine coronavirus. While infection with bovine coronavirus would be expected to be a rare event, the only way to rule it out would be to isolate the virus from infected dogs.

Controlled canine respiratory coronavirus challenge studies in dogs have not been reported to date, and although there appears to be an association between CRCoV infection and canine infectious tracheobronchitis, causality has yet to be definitively confirmed (Erles and Brownlie 2008). Despite this, infection with CRCoV should be considered a differential diagnosis in cases of apparent ‘vaccine failure’ in outbreaks of respiratory disease in fully vaccinated dogs (Ellis et al. 2005). It remains to be seen whether vaccination against CRCoV would confer additional protection against canine infectious tracheobronchitis. Available vaccines containing CCoV are unlikely to provide any protection from disease associated with CRCoV due to significant antigenic dissimilarity (Erles and Brownlie 2005; Kaneshima et al. 2006).

In conclusion, the serological findings described here indicate CRCoV is present in dogs in New Zealand. There is a growing body of evidence suggesting that a number of novel pathogens, including CRCoV, may well play a role in the complex and multifactorial nature of canine infectious tracheobronchitis. Serum antibodies to CIV were not detected in any of the samples collected from dogs, suggesting that this virus is unlikely to be present in dogs in New Zealand.

Declaration of interest

While the study was part funded by Pfizer Animal Health, the authors, two of whom (Knesl and Shields) are employees of Pfizer Animal Health, had full discretion in the study and the content of this paper.

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