Prevalence of antibodies to canine respiratory coronavirus in some dog populations in Japan

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CANINE infectious respiratory disease (CIRD), also known as kennel cough, is a common complex respiratory infection in dogs (Appel and Binn 1987). *Bordetella bronchiseptica*, *Mycoplasma* species, canine parainfluenza virus, canine adenovirus type 2, canine herpesvirus and others have been reported as the causative pathogens (Binn and others 1970, Appel 1981). Erles and others (2003) identified a coronavirus that was serologically and genetically different from the canine coronavirus that causes enteritis and that was closely related to bovine coronavirus (BCoV) and human coronavirus OC43, classified as group 2 coronaviruses in dogs with respiratory signs. They named this virus canine respiratory coronavirus (CRCoV). CRCoV has been suggested to be involved in the manifestation of respiratory signs in dogs (Erles and others 2004), and anti-CRCoV antibodies have been detected in dogs in the USA, Republic of Ireland, UK, Canada, Greece, Italy and Japan, to date (Kaneshima and others 2006, Priestnall and others 2006, Decaro and others 2007). This short communication describes a study to investigate the epidemiology of this virus further, in which Japanese dogs were tested for antibodies to CRCoV.

Serum samples from 85 unimmunised specific pathogen-free (SPF) beagles were examined to determine the cut-off value of the anti-CRCoV antibody test used in this study.

Antibodies to CRCoV were tested by a modification of a previously reported ELISA (Erles and others 2003, Decaro and others 2007). The culture fluid of Mardin-Darby bovine kidney (MDBK) cells (American Type Culture Collection) that were infected with the Mebus strain of BCoV (Stair and others 1972) was concentrated by the addition of ammonium sulphate (Sigma) (Tajima and others 1998). The precipitate was dialysed to phosphate-buffered saline (PBS) and used as the positive antigen. The negative antigen was prepared in the same way from uninfected MDBK cells. Serum diluted 1:50 was added into both the positive and negative antigen-coated wells of a 96-well ELISA plate (Thermo Labsystems). After incubation, the plate was washed with PBS-Tween20 (MP Biomedicals) and peroxidase-conjugated rabbit anti-dog immunoglobulin G (H+L) (Jackson Immuno Research Laboratories) diluted 1:2500 was added to the wells. After incubation, the plate was washed with PBS-Tween20, and substrate (0·05M citric acid containing 0·2mM ABTS diammonium salt [Wako Pure Chemical] and 0·004 per cent hydrogen-peroxide) was added to each well. After incubation, the optical density (OD) was determined at 405 nm. The ELISA value was calculated as follows: ELISA value = OD value of positive antigen-coated well – OD value of negative antigen-coated well.

The mean (sd) of the ELISA values in sera from the SPF beagles was 0·019 (0·023) with the highest value being 0·060. In accordance with a previous report by Decaro and others (2007), the cut-off value was set at the mean plus three sd of the ELISA values of the SPF beagles (0·088).

Sera obtained from 373 clinically healthy dogs that visited veterinary clinics for the purpose of regular vaccination in June to August 2004, from 225 that visited veterinary clinics for vaccination in April to December 1994, and from 90 dogs with respiratory signs and 37 dogs with diarrhoea, all collected in 2004, were examined to investigate the prevalence of anti-CRCoV antibodies in privately owned family pet dogs.

TABLE 1: Prevalence of antibodies to canine respiratory coronavirus in populations of pet dogs in Japan, grouped by sex and pedigree status

	Number positive/number sampled (%) 2004	1994
Sex		
Male	58/204 (28·4)	31/127 (24·4)
Female	35/169 (20·7)	21/98 (21·4)
Pedigree status		
Pedigree	85/328 (25·9)	44/188 (23·4)
Non-pedigree	8/45 (17·8)	8/37 (21·6)

TABLE 2: Prevalence of antibodies to canine respiratory coronavirus in pet dogs with respiratory signs or diarrhoea, and in healthy dogs

Signs	Number of Samples tested	Positive (%)
Respiratory	90	43 (47·8)
Diarrhoea	37	11 (29·7)
Healthy*	373	93 (24·9)

* Samples from the healthy pet dogs in 2004

The seropositivity was analysed by chi-squared test; $P<0·05$ was considered statistically significant.

When the cut-off value or more was regarded as positive, the seropositivity of the healthy dogs sampled in 2004 was 24·9 per cent (93 of 373 positive) and the seropositivity of the dogs sampled in 1994 was 23·1 per cent (52 of 225). There was a trend towards higher levels of seropositivity in male and pedigree dogs, although the increases were not statistically significant (Table 1). The dogs with respiratory signs had a significantly higher seropositivity than the healthy dogs ($P<0·0001$, chi-squared=18·24) and the dogs with diarrhoea ($P<0·05$, chi-squared=3·88). There was no significant difference between the seropositivity of the healthy dogs and the dogs with diarrhoea (Table 2).

Erles and others (2004), Kaneshima and others (2006) and Decaro and others (2007) detected anti-CRCoV antibodies in dog sera in 2001/2002, 1998 to 2004 and 2005/2006, respectively. In the present study, anti-CRCoV antibodies were detected in dog sera collected in 1994 (earlier than some of these studies), confirming that CRCoV infections had occurred at least as early as 1994 in Japan. The seropositivity in 1994 was similar to that in 2004. Furthermore, the seropositivity was higher in male and pedigree dogs in both 1994 and 2004. These findings suggest that the prevalence of CRCoV infection in dogs did not markedly change between 1994 and 2004 in Japan. The seropositivity was significantly higher in dogs with respiratory signs than in healthy dogs and dogs with diarrhoea, showing that there is a positive association between seropositivity and respiratory signs in the general population of pet dogs, in addition to its involvement in CIRD in kennels (Erles and others 2003). Further studies are required to identify the clinical signs associated with CRCoV infection in dogs.

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Notes