NOTE *Virology*

A Serological Survey of Canine Respiratory Coronavirus and Canine Influenza Virus in Korean Dogs

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ABSTRACT. The relationship between canine respiratory coronavirus (CRCoV) and canine influenza virus (CIV) seropositivity in dogs in Korea was examined. Sixty-two of the 483 samples (12.8%) were seropositive for CRCoV by indirect fluorescent antibody (IFA) analysis. Nineteen animals were seropositive for CIV by ELISA out of the 385 samples tested. Serum antibodies for both viruses were detected in 6 of the 483 dogs sampled, suggesting that these viruses are present in dogs in Korea. Although the role of CRCoV in canine infectious tracheobronchitis has not been fully elucidated, co-infection with CIV may synergistically worsen respiratory clinical signs and result in more severe canine tracheobronchitis.

KEY WORDS: CIV, CRCoV, CRID.

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Canine respiratory coronavirus (CRCoV) belongs to coronavirus group 2 and is a causative agent of canine infectious respiratory disease (CIRD). CRCoV is a novel pathogen detected in the respiratory tract of dogs suffering from severe respiratory disease but itself is thought only to be associated with subclinical or asymptomatic disease [7]. To date, few CRCoV strains have been isolated from a small number of countries: the United Kingdom (n=2), Japan (n=2), Italy (n=1), and Korea (n=3) [1, 6, 12, 17]. Antibodies against CRCoV are common in canine populations in the United Kingdom (36.2%), Republic of Ireland (30.3%), USA (54.7%), Japan (17.8%), Italy (32.1%), and New Zealand (29%) [4, 9, 10, 13].

In contrast, influenza A virus, a member of the genus orthomyxovirus, causes significant disease in humans, pigs, horses and fowl, and can have a large economic impact [16]. Infections with a new avian-origin canine influenza virus have been associated with the development of acute respiratory disease, and in Korea the virus can spread from dog to dog through contact infection [14, 15]. The rate of avian H3N2 influenza seropositivity in Korean dogs in 2007 was 0.48% (2/419) for pet dogs and 5.1% (16/311) for farmed dogs, with the exception of a mass CIV infection outbreak that occurred on farms [11]. By comparison, in New Zealand 73 of the 251 dogs tested were seropositive for CRCoV, while CIV was not detected [10]. To examine relationship between the seroprevalence of CRCoV and CIV, the levels of CIV and CRCoV seropositivity were examined in 197 farmed dogs and 286 pet dogs using an Ab ELISA kit (Animal Genetics. Inc., Korea) and a CRCoV indirect fluorescent assay (IFA), respectively.

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The serum from pet dogs (n=286) were collected from animals in Seoul city, Gyeonggi and Gyeongbuk province, and supplied by iNtRON Biotechnology Company (Korea) in 2008. Sera from farmed dogs (n=197) were supplied by Animal Genetics Inc. (Korea) and collected from January through December, 2008. Sera from farmed dogs in Jeonbuk province were harvested prior to the CIV outbreak that occurred on some farms.

The sera were tested for the detection of antibodies to the CRCoV using immunofluorescence assay following microtiter virus neutralization (VN) test using human rectal tumor (HRT-18) cells [1]. Briefly, 50 μl of serial 2-fold dilutions of the serum samples were mixed with equal volume of CRCoV-37 strain ($100TCID_{50}$) [1] in microtiter plates for 60 min after which 100 μl fetal calf serum (FCS) free Dulbecco's minimal essential medium (D-MEM) and trypsin (1 μg/ml, GIBCO) was added to each well. The cells were then incubated at 37°C in 5% CO_2 in a humidified atmosphere for 5 days. Following this incubation, the cells were fixed in methanol/acetone (2:1), and reacted with a commercial anti-CRCoV monoclonal antibody (JENO Biotech. Inc., Korea) which bind to the spike protein of CRCoV (1 hr). CRCoV-positive cells were detected using an FITC-conjugated anti-mouse IgG (Kappel). Samples were evaluated for CRCoV IFA positivity at dilutions above 10-fold due to the combined background effects of trypsin and dog serum on the cells.

The 483 serum samples described above were analyzed for virus-specific antibodies using commercial competitive ELISA assays (Animal Genetics, Inc., Korea) that can detect anti-nucleoprotein (NP) antibodies. The use of this ELISA for CIV detection has been previously validated [11]. The cut off point for a positive sample was over 50% according to manufacturer's instructions.

Table 1. CRCoV and CIV seropositivity rates in farmed and pet dogs

Dog sera	Province/area	% Seropositive			
		CRCoV IFA	CIV ELISA	CIV HI	CRCoV IFA & CIV ELISA
Farmed dog	Jeonbuk	5.1 (5/98)	50.0 (49/98)[a)]	21.4 (21/98)[a)]	3.1 (3/98)[a)]
	Gyeongam	12.2 (6/49)	6.1 (3/49)	6.1 (3/49)	2.0 (1/49)
	Gyeonbuk	24.0 (12/50)	0.0 (0/50)	0.0 (0/50)	0.0 (0/50)
Pet dog	Seoul	20.0 (29/145)	5.5 (8/145)	4.8 (7/145)	1.4 (2/145)
	Gyeonggi	17.4 (8/46)	6.5 (3/46)	6.5 (3/46)	0.0 (0/46)
	Gyeonbuk	2.1 (2/95)	5.2 (5/95)	5.2 (5/95)	0.0 (0/95)
Total		12.8 (62/483)	4.9 (19/385)	4.7 (18/385)	0.8 (3/385)

[a] Ninety-eight dogs on farms in Jeonbuk province were excluded due to a preceding mass CIV infection.

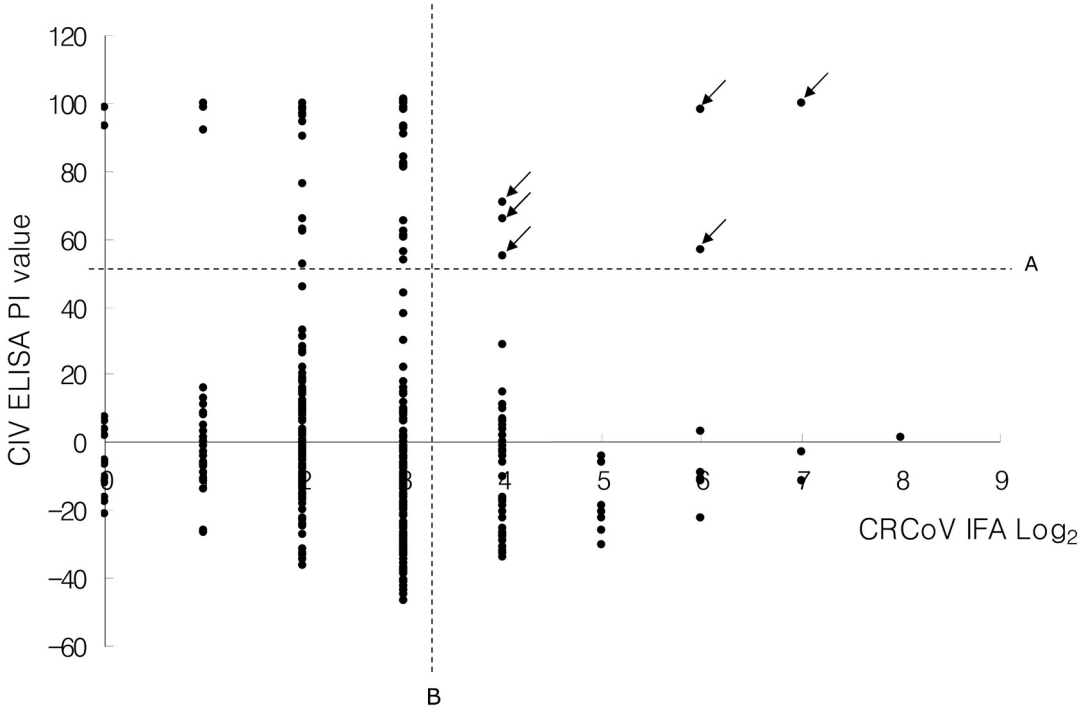

Fig. 1. Antibody seroconversion rates for CRCoV and CIV. CIV antibody titers were regarded as positive if the percent inhibition (PI) value exceeded 50 (A line). Samples were considered positive for CRCoV if antibody could be detected in dilutions greater than 10-fold (B line). The samples that were seropositive for CRCoV and CIV are marked with an arrow.

The samples were also analyzed by a hemagglutination inhibition (HI) test, which measures the ability of the sera to inhibit the hemagglutination activity of a reference virus. The following antigens were used for the HI test: H1N1 influenza virus (A/swine/Korea/GC0503/2005) for H1, H3N2 influenza virus (A/swine/Korea/GC0407/2005) for H3, H5N3 influenza virus (A/duck/Hong Kong/820/1980) for H5, and H9N2 influenza virus (A/chicken/Korea/01310/2001) for H9. The reference viruses were kindly provided by Dr. Dae-Sub Song of Green Cross Veterinary Products (Korea). The HI tests were performed according to procedures recommended by the World Organization for Animal Health (OIE).

Only 62 of the 483 samples tested were positive for CRCoV, indicating a somewhat lower seropositivity in Korea (12.8%) compared to the CRCoV seroprevalence in others countries. Of the 197 farmed dog sera samples, anti-CRCoV antibodies were detected in 23 samples via IFA (11.7%) (Table 1). Different levels of seropositivity were detected in different areas, as 5.1%, 12.2% and 24.0% of the samples from Jeonbuk, Gyeongnam and Gyeongbuk were antibody positive, respectively. Of the 286 pet dog sera, 39 were seropositive, again reflecting different levels in different areas: Seoul (20.0%), Gyeonggi (17.4%), and Gyeongbuk (2.1%) (Table 1). Seropositivity for CRCoV was higher in older dogs than in younger dogs based on a cut-off of two

years of age [10]. However, this study cannot address the effect of age on seropositivity more specifically because precise information correlating sample positivity to animal age was not available.

Nineteen samples of the 385 tested were seropositive for CIV via Ab ELISA (4.9%), excluding the 98 dogs in Jeonbuk province. Of the 286 samples that were collected from pet dogs, 16 were CIV seropositive. This level of seropositivity is higher than previously reported, as only two of 419 samples were found to be positive in 2007 [11]. This result may indicate an increase in CIV outbreaks in pet dogs since 2007.

Importantly, a recent study reported an unprecedented interspecies transmission of a complete avian H3N2 influenza virus to dogs [14]. This is a concern especially in South Korea, where avian influenza viruses (H3N2, H5N1, H6N1, and H9N2) are now circulating or have been detected [3]. Notably, all CIV-positive serum in this study also contained anti-H3 antibodies, consistent with a previous report [11]. The high level of seropositivity in Jeonbuk province may be due to the preceding CIV outbreak in dog farms. In addition, the CIV Ab ELISA detects seropositivity an average of 2 days earlier than the HI test [11]. Although larger field surveys are needed, these data could indicate that the H3 serotype is currently increasing in farmed and pet dogs in Korea.

Several etiological agents are thought to be involved in CIRD, and these generally include CRCoV, canine adenovirus type 1 (CAV-1) and 2 (CAV-2), canine parainfluenza virus (CPiV), canine herpesvirus (CHV), CIV, reoviruses, and *Bordetella bronchiseptica* [2, 5]. CRCoV replication in the respiratory epithelium may damage the mucociliar system and thereby lead to a more severe clinical course of infection than that caused by other respiratory pathogens [2]. CIV-infected dogs develop a distinctively severe and persistent bronchointerstitial pneumonia, which differs from the acute, but short-term, bronchopneumonia that human (H1N1 and H3N2) influenza causes in rodents and ferrets [8].

Serum antibodies to CRCoV and CIV were detected in six of the 483 Korean dogs tested. Of these 6 dogs, 4 were farmed animals and 2 were pet dogs (Fig. 1). Erles *et al.* (2007) previously reported that infection with CRCoV alone caused sub-clinical or mild respiratory symptoms, while CRCoV infection in conjunction with other pathogens resulted in severe respiratory disease and possible exacerbation of disease phenotypes caused by these other pathogenic agents [7]. Additional epidemiological studies are required to determine the exact biological and immunological consequences of co-infection with two emerging viruses in dogs in Korea.

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