

## Serologic Survey for Canine Coronavirus in Adult Dogs

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**Abstract :** The purposes of this study were to survey the seroprevalence of canine coronavirus (CCV) in healthy adult dogs and to determine whether there was any relationship between seroprevalence and the host parameters. Serum samples for determination of serum neutralization antibody titers against CCV were obtained from 812 healthy adult dogs over 1 year old brought to veterinary clinics for routine health care visit in 4 provinces from January 2003 to April 2004. Of the 812 dogs, 714 (87.9%) had positive antibody titers (more than 1 : 4) against CCV. The prevalence of positive CCV antibody titers were not significantly associated with age, sex, rearing province and environment, and vaccination status. However, the positive CCV antibody titers were increasing with the age. These serological findings have shown that prevalence of positive CCV antibody titers in Korean dogs were a relatively high and that CCV infection was widespread in Korean dog population. These suggest that it may be as important to protect dogs against infection with CCV as it is to vaccinate against canine parvovirus.

**Key words :** Canine coronavirus, SN titer, adult dogs, Korea.

### Introduction

Canine coronavirus (CCV) was first isolated from a case of canine enteritis during an epizootic in Germany in 1971 (5), although serological evidence that dogs may be naturally infected with a coronavirus related to transmissible gastroenteritis virus was obtained earlier (6,15). CCV belongs to one of the major antigenic groups of coronaviruses (24,25) and is serologically related to feline infectious peritonitis virus (FIPV), feline enteric coronavirus (FECV), transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus (PRCV) (23). These viruses have been distinguished mainly by their host species of origin. It has been reported, however, that some strains of CCV can also infect cat (4,26) and swine (28). Likewise TGEV can also infect the other member species (15,29) and FIPV can infect swine (28). This close relationship indicates that the viruses may have a common ancestor (8,23).

The disease caused by CCV has not been adequately investigated and that the virus plays in canine enteric illness has still not been well established. In the last decade, as a consequence of the relatively high mutation frequency of RNA positive stranded viruses, CCV has evolved and a new genotype has been identified in the feces of infected dogs (17,22). The several studies carried out by different researchers have focused upon the epidemiological relevance of these viruses and identified the wide diffusion of CCV infections among dog populations (2,7,21).

Serological investigations carried out on a large number of

dogs suggest that CCV infection is widespread (13,16,30). The prevalence of CCV antibodies in different dog populations throughout the world has been found to range from 0 to 80%, with as high as 100% reported in a commercial breeding colony (20,27).

Although CCV was first studied in Korea in the early 1990s (11,31), the extent to which CCV is important as a gastroenteric pathogen in the Korean dog population is presently unknown. And the antigenic and pathogenic properties of CCV strain in Korea were also not studied in detail. Furthermore, it is not clear how many widespread CCV infection is in dogs in Korea nowadays. The purposes of this study were to survey the seroprevalence of CCV in healthy adult dogs and to determine whether there was any relationship between seroprevalence and the host parameters.

### Materials and Methods

#### Samples and data collection

Serum samples for determination of CCV antibody titers were obtained from 812 healthy adult dogs over 1 year old brought to veterinary clinics for routine health care visit in 4 province (Gangwon, Gyeonggi, Seoul, and Others) from January 2003 to April 2004. Details on the sex, age, breed, rearing environment and province and vaccination against CCV were recorded. Sera obtained by the centrifugation of clotted blood were stored at  $-20^{\circ}\text{C}$  until used.

#### Serum neutralization (SN) test

Serum CCV antibody titer was determined by the SN test as described by Barlough *et al* (3), with minor modification.

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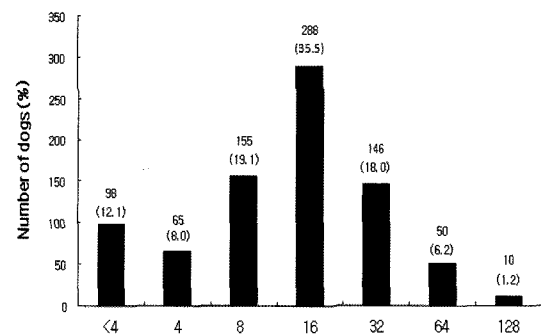
Prior to use, all test sera were heated to 56°C for 30 minutes in order to inactivate heat-labile virus inhibitors. For each individual sample, serial two fold dilutions of the sera were mixed with an equal volume of an K378 strain CCV suspension containing approximately 200 TCID<sub>50</sub>/0.1 ml and the mixtures were incubated at 37°C for 60 minutes. Each mixture was then inoculated into the 1 × 10<sup>5</sup> A-72 cell cultures in flat bottomed microplates (Corning, USA), and incubation was made in an atmosphere of 5% CO<sub>2</sub> in air at 37°C for 3 to 5 days. In each test, known negative and positive sera with moderate and high titers were included. All SN tests were performed in duplicate. The antibody titer was expressed as the reciprocal of the highest dilution of the test serum that completely inhibited the viral cytopathic effect (9,10). The titer more than 1:4 was regarded as antibody-positive in this study (12).

### Statistical analysis

Serological test result and potential associations with age, sex, breed, rearing environment and province, primary vaccination, and revaccination were assessed by the use of a Chi-square test and Cochran-Mantel-Haenszel trend test. All of the analyses described above were performed using SAS version 9.1 (SAS Institute, USA). *P* values less than 0.05 were considered significant, if not indicated otherwise.

## Results

**SN titers :** SN antibody titers against CCV of 812 adult dogs were evaluated. Of the 812 dogs, 714 (87.9%) had positive antibody titers (more than 1 : 4) against CCV, and 98 (12.1%) had less than positive titers (Fig 1).



**Fig 1.** Distribution of dogs (n = 812) by serum neutralization antibody titers against canine coronavirus. Parentheses indicate percentage.

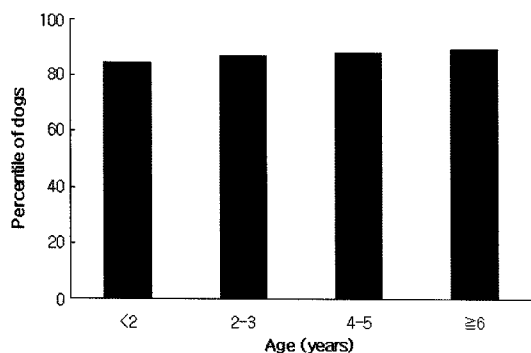
**Age :** One hundred twenty three (84.8%) of 145 dogs in less than 2 years of age had positive antibody titers, 257 (87.4%) of 294 dogs in between 2 and 3 years of age had positive CCV antibody titers, 138 (88.5%) of 156 in between 4 and 5 years of age had positive antibody titers, 129 (88.4%) of 146 in between 6 and 8 years of age had positive antibody titers, and 67 (94.0%) of 71 in older than 8 years of age had positive antibody titers. The dogs with positive CCV antibody titers were increasing with the age. But, the prevalence of positive CCV antibody titers were not significantly associated with age-group (Fig 2). However, in Cochran-Mantel-Haenszel trend assay antibody titers of dogs against CCV were significantly associated with the age (*P*=0.0027).

**Breed :** Of the 812 dogs, 748 (92.1%) dogs were purebred and 64 (7.9%) dogs were mixed breed. The prevalence of positive antibody titers by breeds were varied (Table 1).

**Sex :** Three hundreds and sixty seven dogs (45.2%) were

**Table 1.** Dogs with serum neutralization antibody titers of each breed against canine coronavirus

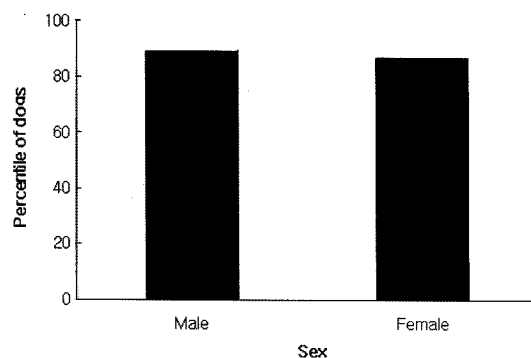
Breed	No. of dogs	No. of dogs with positive ( $\geq 1 : 4$ ) SN titer (%)	No. of dogs with under positive ( $< 1 : 4$ ) SN titer (%)
Shih-tzu	125	105(84.0)	20(16.0)
German shepherd dog	113	104(92.0)	9(8.0)
Maltese	110	101(91.8)	9(8.2)
Yorkshire terrier	87	71(81.6)	16(18.4)
Cocker spaniel	65	57(87.7)	8(12.3)
Poodle	53	48(90.6)	5(9.4)
Miniature schnauzer	31	27(90.3)	3(9.7)
Jindo dog	25	20(80.0)	5(20.0)
Pekingese	16	13(81.2)	3(18.8)
Pomeranian	14	13(92.9)	1(7.1)
Golden retriever	13	10(76.9)	3(23.1)
Miniature pinscher	11	8(72.7)	3(27.3)
Alaskan malamute	10	10(100)	0(0)
Other purebred	75	65(86.7)	10(13.3)
Mixed breed	64	61(95.3)	3(4.7)
Total	812	714(87.9)	98(12.1)



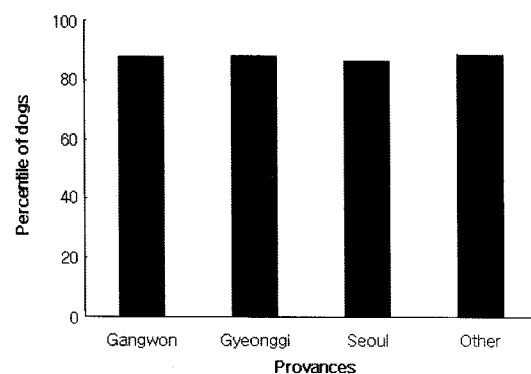
**Fig 2.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by age.

male and 432 dogs (53.2%) were female, and 13 dogs (1.6%) were not recorded on sex. 327 (89.1%) of 367 male dogs and 375 (86.8%) of 432 female dogs had positive antibody titers. Positive CCV antibody titers were not significantly associated with sex (Fig 3).

**Rearing province :** Province was classified into Gangwon, Gyeonggi, Seoul and other provinces (Gyeongbuk, Gyeongnam and Jeju). Overall, 303 (88%) of 344 dogs in Gangwon, 123 (88%) of 139 in Gyeonggi, 198 (86.8%) of 227 in Seoul, and



**Fig 3.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by sex.

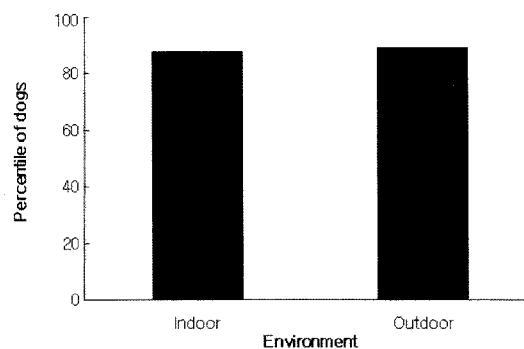


**Fig 4.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by rearing province.

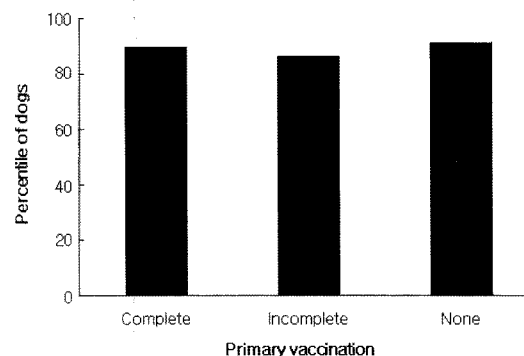
90 (89%) of 101 in other provinces had positive antibody titers. There was no statistical difference on positive CCV antibody titers among the rearing provinces (Fig 4).

**Rearing environment :** Four hundreds and eighty four dogs (59.6%) were reared in indoor and 248 dogs (30.5%) in outdoor, and 80 dogs (9.9%) were not recorded. Overall, 424 (87.6%) of 484 indoor dogs and 221 (89.1%) of 248 outdoor dogs had antibody titers higher than the positive titer. The positive antibody titer were not significantly different between the rearing environment (Fig 5).

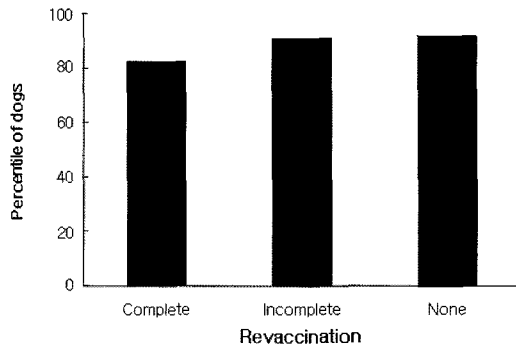
**Vaccination history :** Primary vaccination histories for 657 (80.9%) out of 812 dogs were available. Of the dogs for which primary vaccination history were known, 588 (89.5%) dogs had positive antibody titers. The 532 (89.6%) of 594 dogs in the complete primary vaccination group had positive antibody titers and 25 of 29 (86.2%) dogs in the incomplete primary vaccination group had positive antibody titers. Thirty one (91.2%) of 34 dogs in the none primary vaccination group had serum CCV antibody titer higher than the positive titer. There were no significant difference on the prevalence of CCV antibody titers among the primary vaccination groups (Fig 6). Of the 812 dogs, revaccination history was recorded in 633 (77.9%) dogs. One hundred and fifty (82.4%) of 182



**Fig 5.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by rearing environment.



**Fig 6.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by primary vaccination (complete, more than three times vaccinated; incomplete, less than three times vaccinated; and none, unvaccinated).



**Fig 7.** Distribution of dogs with positive serum neutralization antibody titers against canine coronavirus by revaccination (complete, annually revaccinated; incomplete, intermittently revaccinated; and none, unvaccinated).

in the complete revaccination group, 168 (90.8%) of 185 in the incomplete revaccination group, and 244 (91.7%) of 266 in the none revaccination group had higher than positive CCV antibody titers. Moreover, The positive serum CCV antibody titers were not statically different among the revaccination groups (Fig 7).

## Discussion

Measuring serum antibody titers in companion animal is to provide a rational way of establishing whether an animal has had a protective antibody response to a specific disease agent and to use as a epidemiology of the infection (19). Puppies become immune to re-infection after recovery from CCV infection. As SN antibodies alone appear not to protect dogs from CCV infection, it is therefore likely that local mucosal immunity is more important (1,27).

Recently serological investigations carried out on a large number of dogs suggest that CCV infection is widespread in populations where seroprevalence may be particularly high (approximately 70-90%) (16,19,30). Seroprevalence of CCV infection in each countries had been reported to be 15.8% in Australia (14), 90.8% in Italy (16), 44.1% in Japan (2), 76% in England (27), and 74.3% in Turkey (30). In the previous studies conducted in Korea, the seroprevalences of CCV infection were 52.5% (31) and 48.9%(11). In this study we found that a high percentage (87.9%) of health adult dogs had antibodies against CCV. This high rate of serum positivity was considered by natural active infection and CCV vaccination.

In this study, although prevalence of positive CCV antibody titers were not significantly associated with age, the prevalence of positive CCV antibody titers were increasing with the age. However, in Cochran-Mantel-Haenszel trend assay, antibody titers of dogs against CCV were significantly associated with the age ( $P = 0.0027$ ). In this study, serum CCV antibody titers were not significantly associated with sex. Zarnke *et al* showed that sex were not significantly associated with serum CCV antibody titers (32).

Naylor *et al* (27) showed the prevalence of CCV antibod-

ies was 15.8% dogs in dogs housed singly or in a small groups but a significantly higher prevalence of 40.8% was found among kennelled dogs. The different seroprevalences in the pet dog and kennel populations were probably due to differences between social interactions in the two environments and the greater opportunity for exposure to the virus. However, our results showed that there were no significant difference in seroprevalence between indoor dogs and outdoor dogs, and among the rearing provinces. The similar results were also obtained by Yeilbaet *al* (30) and Zarnke *et al* (32).

Primary vaccination and revaccination status were not significantly associated with positive serum CCV antibody titers. However, complete primary vaccination group had higher positive CCV antibody titer compared to incomplete primary vaccination group. Interestingly, none vaccination group had higher positive CCV antibody titer compared to complete and incomplete vaccination groups. This result may come from the fact that many healthy adult dogs might be recovered from natural infection of CCV and canine parvovirus (CPV) in younger period (13,14,17).

The value of CCV vaccines in providing adequate immunity, under field conditions, is controversial. In a recent study, Pratelli *et al* (18) demonstrated the low efficacy of a widely used inactivated commercial vaccine in reducing fecal shedding after challenge with a field virus. Although the efficacy and the duration of immunity engendered by inactivated vaccines have not been substantiated, it is considered that CCV vaccines are efficacious for increasing positive CCV antibody titers and protecting dogs (18,19).

These serological findings have shown that prevalence of positive CCV antibody titers in Korean dogs were a relatively high and that CCV infection was widespread in Korean dog population. These suggest that it may be as important to protect dogs by vaccination with CCV as it is to vaccinate against CPV.

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## 건강한 성견의 canine coronavirus에 대한 항체가 조사

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**요 약** : 건강한 성견들의 혈청 CCV 항체 역가 보유실태와 역학적인 요인들과 관련성을 조사하기 위하여, 2003년 1월부터 2004년 4월까지 국내의 1세 이상된 성견 812두를 대상으로 CCV에 대한 중화항체 역가를 조사하였으며 연령, 성별, 사육지역, 사육환경 및 기초 예방접종과 추가 예방접종 여부 등의 역학적 요인들 별로 항체보유 상태를 조사하였다. 조사대상 812두의 성견 중 87.9%(714두)가 CCV에 대하여 항체 양성(1:4 이상) 반응을 보였다. 연령, 성별, 사육지역, 사육상태 및 예방접종 상태는 항체보유율에 영향을 미치지 않았으나 나이가 증가함에 따라 항체 보유율이 증가하는 양상을 보였다. 이상의 결과를 종합해볼 때, 국내의 성견들은 CCV에 대하여 항체보유율이 대체로 높은 수준이었으며 CCV 감염이 전국에 걸쳐 발생하는 것으로 판단되므로 개들의 CCV에 대한 방어를 위하여 위생적인 관리와 함께 예방접종이 필요한 것으로 생각되었다.

**주요어** : Canine coronavirus, 중화항체가, 성견, 대한민국