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Prevalence of Canine Coronaviral Enteritis in Korea

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Abstract : Canine coronavirus (CCV) is a cause of sporadic outbreaks of enteritis in dogs. This study was performed to carry out epidemiological investigation on the recent outbreaks of CCV enteritis of dogs and determined the potential prognostic factors affecting the survival of dogs. The 131 (34.4%) out of 381 fecal samples collected from dogs with enteritis were positive for CCV by RT-PCR. The fecal samples contained genotype I (30.5%), genotype II (29.0%), and both genotypes (40.5%) of CCV. The majority of dogs with CCV infection ranged 6-18 weeks of age. Age over 18 weeks was significantly associated with higher survival rate ($P < 0.05$). Of the clinical signs examined, dogs without anorexia were significantly higher survival rate ($P < 0.01$). The 90.1% of dogs with CCV infection were co-infected by CCV and CPV-2. From the results of this study, it can be concluded that CCV infection is widespread in the Korean dog population and CCV may be attributed to be one of the important agents causing enteritis in pups.

Key words **:** Canine coronavirus, enteritis, epidemiology, M gene, Korea.

Introduction

Canine coronavirus (CCV) is a member of the Coronaviridae, a family of viruses belonging to the order Nidovirales (27). CCV was first isolated in 1971 during an epizootic in Germany from a case of canine enteritis (6). CCV is responsible for mild or moderate enteritis in dogs. The morbidity was high, but the mortality rate was low (11). In naturally occurring infections, pups of six to 12 weeks old appear to be more susceptible to disease. This may be related to the decline in maternal antibody (7,31).

Common clinical signs are soft feces or fluid diarrhea, vomiting, dehydration, loss of appetite and, occasionally, death. Although adult dogs generally have no or mild clinical signs and usually recover after a brief period of illness, pups with secondary bacterial infections, parasites, or other viruses may suffer severe, even fatal disease. Co-infections by CCV and canine parvovirus type 2 (CPV-2) and/or other pathogens are especially severe when infections occur simultaneously (1,11).

The virus is difficult to isolate by cell culture (17) and to detect by electron microscopy (EM) of coronavirus like particles in stools. These problems in isolating CCV still hinder studies on their epidemiology and pathogenesis (19). Recently, reverse transcriptase polymerase chain reaction (RT-PCR) and nested polymerase chain reaction (n-PCR) on a fragment of the gene encoding for the M protein was developed for the diagnosis of CCV infection to overcome the difficulties in virus isolation in vitro (20).

Detection of CCVs and the findings of novel strains were recently reported (15,22,33). Two separate clusters of CCVs have been demonstrated in dogs from Italy (22). The first cluster includes CCV intermingled with reference CCV strains (genotype II), such as Insavc-I and K378, while the second cluster segregates separately from CCV and, presumably, represents a genetic outlier referred to as FCoV-like CCV (genotype I) (23).

In Korea, CCV infections in dogs have been reported from the 1990' (13,16,34). However, the epidemiological study on dogs with CCV infection has not been yet reported. The objectives of present study were to perform the epidemiological investigation on the recent outbreaks of CCV enteritis of dogs and to determine the potential prognostic factors affecting the survival of dogs.

Materials and Methods

Clinical samples and data collection

The total of 381 fecal samples of dogs with enteritis signs were collected by local veterinarians in Chungbuk, Chungnam, Gangwon, Gyeonggi, Gyeongbuk, Gyeongnam, Jeonbuk, Jeonnam, Jeju, and Seoul area between June, 2003 and September, 2007. Serum samples were taken from 358 dogs for serological examination. Details on the sex, age, vaccination status and breed were recorded. Vaccination status was recorded according to the notification by owner of the patient. Fecal specimens were taken by swab from the anus and homogenized (10% w/v) in phosphate buffered saline (PBS, pH 7.2).

RT-PCR and nested PCR assay

Viral RNA was extracted from each fecal specimen using the RNeasy mini kit (Qiagen GmbH, Germany). The RT-PCR

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assay was carried out as previously reported with slight modification (21).

The 100-fold diluted products of first round PCR were subjected to a nested PCR for genotyping using the CCV2 and CCV1a (5'-GTG CTT CCT GAA GGT ACA-3') primers for genotype I, and CCV2 and CCV3 (5'-GGT GTC ACT CTA ACA TTG CCT-3') primers for CCV genotype II (18). Amplication reaction for nested PCR was same as RT-PCR.

Confirmation of CPV by PCR

To confirm the CPV infection, the PCR was carried out with a minor modification of our previous protocol to detect VP2 gene from the fecal swab samples (12).

Serum neutralization (SN) test

Serum CCV antibody titer was determined by the SN test as described by Barlough *et al* (4), with minor modification. A-72 cells were propagated in Dulbecco modified Eagle's medium that contained 10% fetal bovine serum and antibiotics (at final concentrations of penicillin 5000 IU/ml, streptomycin 2500 μg/ml, and amphotericin B 10 μg/ml).

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 (isolated in USA and classified as genotype II) CCV suspension containing approximately 200 $TCID_{50}$/0.1 ml and the mixtures were incubated at 37°C for 60 minutes. Each mixture was then inoculated into the 1×10^5 A-72 cell cultures in flat bottomed microplates (Corning, USA), and incubation was made in an atmosphere of 5% CO_2 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 (21,30). In this study, titer more than 1:4 was regarded as antibody-positive as suggested by Keenan *et al* (11) and Tennant *et al* (30).

Statistical analysis

A two-tailed Fisher's exact test or chi-square test was used to evaluate the hypothesis that survival rate was homogenous among dogs according to the study factors. Data were summarized by descriptive statistics. Relationship between survival rate and independent variables (age, sex, season, vaccination statues, survival, SN titer, and clinical signs) was evaluated by unconditional logistic regression (live = 0, death = 1) by creating dummy variables for n variables with n-1 levels each. All independent variables were initially screened by univariate analysis to assess simple association between survival and independent variables by calculating odds ratios (ORs), associated 95% confidence intervals (95% CI), and P values. Univariable association between the independent variables and survival firstly were determined at $P < 0.20$. Selection of variables for multivariable modeling with a forward stepwise analysis was performed as described by Hosmer and Lemeshow (10). Interaction terms were not considered to simplify the model interpretation. The multivariate modeling approach allows to evaluate several factors simultaneously while controlling for potential confounding factors. This statistics was calculated for the final model to evaluate the goodness-of-fit of the data. All analyses were performed using SAS version 9.1 (SAS institute, Cary, NC). P values less than 0.05 were considered statistically significant, if not indicated otherwise.

Results

CCV confirmed by RT-PCR

Overall, 131 (34.3%) out of 381 dogs with enteritis were diagnosed as CCV enteritis by RT-PCR. Of these 131 dogs, 118 (90.1%) showed co-infection by CCV and CPV-2, and 13 (9.9%) showed CCV infection alone (Table 1).

Genotypes of CCV

CCV positive samples were further subjected nested PCR for genotyping. Forty (30.5%) samples were positive for genotype I, and thirty eight (29.0%) for genotype II. In 53 (40.5%) samples, both genotypes were detected (Table 1).

Distribution of risk factors

Only eighty-six out of 131 dogs with CCV enteritis were statistically analysed because vaccination history and other demographic information were not available across all dogs (Table 2). The prevalence of CCV enteritis of male dogs (55.8%) were higher than that of female dogs (44.2%). The overall survival rate of dogs infected with CCV was 55.8% and survival rates of female dogs (63.2%) were higher than that of male dogs (50.0%). However, there were not statistical difference in prevalence or survival rates between male and female.

Three (3.5%) dogs were less than 6 weeks of age, 67 (77.9%) dogs were between 6 and 18 weeks of age, 7 (8.1%) dogs were more than 19 and 51 weeks of age, and 9 (10.5%) dogs were older than 1 year. Overall, 81.4% dogs were less than 19 weeks of age. In death rate according to age, the younger dogs had the higher death rate.

Thirty seven (43.0%) dogs were not vaccinated, 31 (36.1%) dogs were incompletely vaccinated, and 18 (20.9%) dogs

Table 1. Genotypic distribution of dogs (n = 131) with canine coronavirus enteritis and co-infection by CCV and CPV-2

CCV	No. of dogs (%)
CCV genotype I *	40 (30.5)
CCV genotype II **	38 (29.0)
CCV genotype I and II	53 (40.5)
Co-infection by CCV and CPV-2	118 (90.1)

*CCV genotype I; FCoV-like CCV strain.
**CCV genotype II; typical CCV strain.

Table 2. Distribution of survival in dogs (n=86) with canine coronavirus enteritis by risk factors

Variable		No. of dogs (%)	No. survived (%)
Sex	Male	48 (55.8)	24 (50.0)
	Female	38 (44.2)	24 (63.2)
Age (weeks)	< 6	3 (3.5)	1 (33.3)
	6- ≤ 18	67 (77.9)	34 (59.6)
	19- ≤ 51	7 (8.1)	5 (71.4)
	≥ 52	9 (10.5)	8 (88.9)
Vaccination	Complete	18 (20.9)	14 (77.8)
	Incomplete	31 (36.1)	16 (51.6)
	None	37 (43.0)	18 (48.6)
SN titer	< 4	17 (19.8)	8 (47.1)
	4	21 (24.4)	8 (38.1)
	8	33 (38.4)	21 (63.6)
	16	14 (16.3)	11 (78.6)
	32	1 (1.1)	0 (0.0)
Clinical signs	Diarrhea	38 (44.1)	2 (5.3)
	Vomiting	46 (53.5)	23 (50.0)
	Anorexia	59 (68.6)	28 (47.5)
	Respiratory sign	15 (17.4)	8 (53.3)
Season	Spring	25 (29.1)	14 (56.0)
	Summer	14 (16.3)	7 (50.0)
	Autumn	19 (22.1)	9 (47.4)
	Winter	28 (32.6)	18 (64.3)
Co-infection	Only CCV	5 (5.8)	3 (60.0)
	CCV and CPV-2	81 (94.2)	45 (55.6)

Table 3. Breed distribution of the dogs with canine coronavirus enteritis

Breed	No. of dogs	%
Shih-tzu	14	16.3
Maltese	11	12.8
Alaskan malamute	9	10.5
Cocker spaniel	6	7.0
Mixed	6	7.0
German shepherds	4	4.7
Siberian husky	4	4.7
Jindo	4	4.7
Retriever	3	3.5
Poodle	3	3.5
Yorkshire terrier	3	3.5
Pekinese	2	2.3
Pomeranian	2	2.3
Rottweiler	2	2.3
Samoyed	2	2.3
Schnauzer	2	2.3
Beagle	2	2.3
Chow Chow	1	1.2
Pungsan dog	1	1.2
Great pyrenees	1	1.2
Chihuahua	1	1.2
Pit bull terrier	1	1.2
Pembroke welsh corgi	1	1.2
Peruvian hairless dog	1	1.2
Total	86	100

were completely vaccinated. The dogs vaccinated completely had higher survival rate than dogs incompletely vaccinated or unvaccinated. Seventeen (19.8%) of 86 dogs had less than titer 1:4, Sixty-nine (80.2%) dogs had positive titers (more than 1:4).

The prevalence of the clinical CCV infection during winter (32.6%) and spring (29.1%) was higher than those of autumn (22.1%) and summer (16.3%). Seventy nine dogs (91.9%) of 86 dogs showed watery or hemorrhagic diarrhea, and 46 (53.5%) dogs showed vomiting. Fifty nine (68.6%) of 86 dogs showed anorexia, and 15 (17.4%) dogs showed respiratory signs.

The most prevalent breed was Shih-tzu (16.3%) and then the prevalence followed by Maltese (12.8%), Alaskan malamute (10.5%), Cocker spaniel and Mixed (7.0%), German shepherd and Hursky (4.7%) (Table 3).

Risks factors associated with survival

The results of unconditional logistic regression analysis of the relationship between independent study variables and survival are summarized in Table 4. Survival rate of complete vaccinated group was significantly higher than that of non-vaccinated group, but not in incomplete group. When entering variable with $P < 0.20$ into the final model, three variables, anorexia, vomiting and age, were significantly associated with the survival: dogs without anorexia and vomiting, or age over 18 weeks were found to have higher survival rate. In the final logistic regression model, vaccination status and vomiting were no longer significantly associated with the survival (Table 5).

Discussion

CCV is a cause of sporadic outbreaks of enteritis in dogs. CCV is now recognized as an important, but imperfectly understood, pathogen of dogs. CCV appears to be enzootic worldwide since the virus has been demonstrated in many countries. The seroprevalence of CCV in pet dogs is reported to range from 6 to 75 per cent and in kennel populations the seroprevalence may reach 80 per cent (25,29). The seroprevalence of CCV in Korean dogs was reported to 42 per cent in 1988 and 55 per cent in 1993 (13).

Although commercial vaccines have been used to prevent CCV infection from early 1990's in Korea, outbreaks of CCV

Table 4. Unconditional logistic regression analysis of the relationship between the study variables and the survival by canine coronavirus enteritis

Variable		No. survived / No. cases	OR* (95% CI)	*P*
Sex	Male	81 / 114	1.0	
	Female	65 / 102	1.46 (0.84-2.53)	0.1827
Age group (weeks)	< 18	86 / 156	1.0	
	≥ 18	48 / 60	2.87 (1.44-5.72)	0.0028
Vaccination status	None	26 / 53	1.0	
	Incomplete	44 / 72	1.63 (0.8-3.34)	0.1809
	Complete	64 / 91	2.46 (1.22-4.97)	0.0119
SN titer	< 1:4	27 / 48	1.0	
	≥ 1:4	107 / 168	1.36 (0.71-2.62)	0.3498
Season	Spring	19 / 35	0.76 (0.33-1.77)	0.5224
	Summer	35 / 55	1.12 (0.52-2.39)	0.7731
	Autumn	44 / 67	1.22 (0.59-2.53)	0.5883
	Winter	36 / 59	1.0	
Clinical signs				
Anorexia	Yes	92 / 157	0.27 (0.12-0.59)	0.0010
	No	42 / 59	1.0	
Diarrhea	Yes	69 / 144	0.43 (0.18-1.04)	0.0620
	No	24 / 72	1.0	
Vomiting	Yes	71 / 131	0.45 (0.25-0.82)	0.0084
	No	62 / 85	1.0	
Respiratory signs	Yes	28 / 40	1.54 (0.74-3.23)	0.2526
	No	106 / 176	1.0	
Co-infection by CCV and CPV-2	No	8 / 12	1.0	
	Yes	126 /204	0.8 (0.23-2.77)	0.7342

*The 95% confidence interval of the odds ratio (OR) was calculated as the antilog (coefficient ± 1.96 × SE of the coefficient).

Table 5. Odd ratios (ORs) and 95% confidence intervals (CIs) of predictor variables associated with the survival by canine coronaviral enteritis in the final logistic regression model

Variable		No. survived / No. cases	OR (95% CI)	*P*
Anorexia	Yes	92 / 157	0.27(0.12-0.59)	0.0012
	No	42 / 59	1.0	
Age group (weeks)	< 18	86 / 156	1.0	
	≥ 18	48 / 60	2.88 (1.42-5.83)	0.0033

enteritis continued. This study was aimed to detect CCV infection and to determine the relation between the epidemiological parameter and the recent outbreaks, and to determine the potential prognostic factors affecting the survival of dogs.

In this study, identification of CCV RNA-genome was successfully achieved using the RT-PCR method with set of M gene specific primers. The author determined the presence of the M gene in 131 (35.5%) of the 381 dogs with clinical signs of enteritis. The detection rate of this study was lower than 57.3% in Japan (14) and 64.9% in Italy (22). However, the detection rate of in Korea was higher than that (12.8%) in Thailand (26).

There are two genotypes of CCV. The first type (genotype II) includes CCV intermingled with reference CCV strains, such as Insavc-1 and K378, while the second type segregates separately from CCV and presumably represents a genetic outlier referred to as FCoV-like (genotype I) CCV segregating clearly from the typical strains and is more closely related to FCoV strians than to CCV strains. It appears that some strains of FCoV-like CCV may be more virulent than typical CCV strains, causing severe hemorrhagic diarrhea. (5,17,19, 22). In this study, the 69.5% of dogs with CCV enteritis resulted positive for genotype I and 71.0% for genotype II. In other countries, CCV genotype I of infection has been reported

to be 14.5% (24) and 45.5% (8) in Italian dogs and 47.5% (32) in China foxes, but CCV genotype I was not detected in several China city dogs (32). Analogously, CCV genotype II has been reported to be 8.7% (24) and 28.4% (8) in Italy and 86.4% (32) in China. In this study, the 40.5% of dogs with CCV enteritis were infected with both genotype I and II, showing that dual CCV infections were very frequent in the Korean dog population. As reported previously, dual CCV infection has been 76.8% (24) and 23.3% (8) in Italy, 40.9% in China foxes, and 66.6% in China raccoon dogs (32).

In this study, the overall survival rate of dogs infected with CCV was 55.8% and the prevalence of CCV enteritis between male dogs and female dogs was not different. It has been reported that sex was not a significant factor to CCV enteritis prevalence (35).

In general, six- to 12-week-old pups appear to be more susceptible to CCV enteritis possibly in association with the decline of their maternal antibodies (7). In this study, CCV positive dogs had various age from 4 week to 2 years old, but majority (77.9%) of CCV enteritis dogs were from 6 to 18 weeks of age in which was a period for vaccination. In other studies on the significant factors for CCV and FCoV infection, there was no significant difference between the age and CCV infection (35). However, there was statistically significant difference between age and infection of CCV (29).

The incidence of clinical CCV disease in domestic dogs increases in winter months (28). Virus shed in dog feces during winter months survives longer than virus shed during summer months, presumably due to lower temperatures and lower ultraviolet exposure in winter (7,30). In this study, CCV enteritis in dogs was more prevalent in winter (32.5%) and spring (29.1%), but there was no significant difference in prevalence of CCV infection among seasons. Owners should be encouraged to have their dogs vaccinated prior to the high risk season, particularly if exposure to other dogs is expected.

Clinical sign of dogs with CCV enteritis in this study were variable. The majority of dogs had a history of diarrhea (91.9%), anorexia (68.6%), and vomiting (53.5%). Dog with diarrhea, vomiting, and respiratory sign were not significantly associated with survival rate. However, there was significant difference in survival rate with anorexia sign. The persistence and severity of clinical signs can often be related to stress and the presence of other pathogens such as CPV-2 (2,3,7). In this study, 118 (90.1%) of 131 dogs were co-infection by CCV and CPV-2. Co-infection is very frequent in the dog population, as reported previously (2,14,26). This led to experimental studies that demonstrated the severity of clinical signs, and the signs were more pronounced in dogs that had both CCV and CPV-2 infections compared with CPV-2 alone (2). However, in this study, co-infection were not significantly associated with survival rate.

In this study, 79% of dogs with CCV enteritis were not vaccinated or incompletely vaccinated and the most of these dogs were under 18 weeks of age. However, the 20.9% of dogs with CCV enteritis were completely (more than twice) vaccinated. The 80.2% of dogs with CCV enteritis had SN titer over 1:4 that is more than positive titer level. It is considered that serum antibody does not prevent outbreaks of CCV enteritis. And it appears that local immunity (IgA in the gut mucosa) is essential for protection against CCV infection (1).

From the results of this study, it can be concluded that CCV infection is widespread in the Korean dog population, and CCV may be attributed to one of the important agents causing diarrhea in pups. Prophylactic measures should focus on the isolation of susceptible dogs from the dog population until the vaccination can be expected to reach the appropriate level of protection.

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개 코로나바이러스성 장염의 발생 역학

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요 약 : 본 연구는 최근 국내에서 발생하는 CCV 장염의 역학적 특성을 조사하기 위하여, 2003년 6월부터 2007년 9월까지 전국의 10개 지역 동물병원에서 설사증상을 보이는 381두의 분변 시료를 채취하였으며, 이중 358두는 혈청학적 검사를 위하여 혈청 시료를 채취하였다. 또한 CCV 장염의 발생에 대한 역학적 조사와 CCV 장염에 이환된 환자의 생존에 영향을 미치는 역학적 요인들에 대하여 조사하였다. 설사증상을 보이는 총 381두중 131두(34.4%)가 CCV 장염으로 확진되었으며, 그 중 30.5%의 변에서는 genotype I이 검출되었고 29.0%에서는 genotype II가 검출되었으며 40.5%에서는 genotype I과 II가 혼합 감염되어 있었다. CCV 장염은 6-18주령의 예방접종을 실시하지 않았거나 불완전하게 실시한 개체에서 주로 발생하였다. 주요 임상증상으로 설사, 구토와 식욕 결핍 증상이 대부분의 환축에서 나타났다. CCV에 이환된 환자 중 나이가 많거나 예방접종을 완료했을 경우 생존율이 높았으며($P < 0.05$) 식욕 결핍이 없는 개체일수록 생존율이 높게 나타났다($P < 0.01$). CCV 장염에 이환된 환자의 대부분은 개 파보바이러스(CPV-2)와 혼합감염되었으나 이로 인한 생존율에서 유의차는 나타나지 않았다. 따라서 국내에서 CCV의 감염은 빈번하게 발생하고 있으며, CCV 장염은 어린 개체에서 설사를 일으키는 중요한 요인 중의 하나로 확인되었으므로 CCV 감염된 집단으로부터 감수성 있는 개체를 분리하고 CCV에 대한 예방접종을 실시할 필요성이 제기되고 있다.

주요어 : Canine coronavirus, enteritis, epidemiology, M gene, Korea