Journal of Bacteriology and Virology 2010. Vol. 40, No. 2 p.77 – 81
DOI 10.4167/jbv.2010.40.2.77

Original Article

Serological Survey for Canine Parvovirus Type 2a (CPV-2a) in the Stray Dogs in South Korea

Dong-Kun Yang*, Soon-Seek Yoon, Jae-Won Byun, Kyung-Woo Lee, Yoon-I Oh and Jae-Young Song

National Veterinary Research and Quarantine Service, MIFAFF, Anyang, Korea

Canine parvovirus type-2 (CPV-2) is one of the major diarrhea-causing agents, inducing acute hemorrhagic gastroenteritis in puppies. In this study, we conducted a seroepidemiological survey of CPV-2a in stray dogs in South Korea. In total, 405 canine sera, collected between 2006 and 2007, were screened for the presence of antibodies against CPV-2a using a hemagglutination inhibition (HI) assay. The positive rate in stray dogs tested for CPV-2a was 93.8%. The regional CPV-2a prevalence was 100% (8/8) in Jeju, 95.1% (232/244) in Gyeonggi, 94.7% (36/38) in Jeonra, 92.9% (13/14) in Gangwon, 92.7% (38/41) in Chungcheong, and 88.3% (53/60) in Gyeungsang province. No significant difference in the seropositive rate was found between male (93.6%) and female (94.0%) dogs. Analysis of the distribution of HI titer against CPV-2a according to the age of the stray dogs showed a linear increase in seroprevalence with age, although the association with age was not statistically significant. The incidence of stray dogs showing an HI antibody titer above 1:5120 was estimated to be 26.2%. Thus, the presence of high HI antibody against CPV-2a may indicate circulation of CPV-2a in stray dogs.

Key Words: Canine parvovirus type-2, Seroepidemiological survey, Stray dog, HI assay

INTRODUCTION

Canine parvovirus (CPV) infection is one of the most important viral diseases in dogs. CPV causes disease characterized by enteritis, leukopenia, nausea, and myocarditis in puppies over the age of 2 months (1, 2). CPV belongs to the family of *Parvoviridae*, genus *Parvovirus*, along with the feline panleukopenia virus, and possesses a negative single-strand DNA genome (3). Since a new CPV was first identified in the late 1970s in dogs, this CPV has spread worldwide and has been named as CPV-2, to distinguish it from the CPV prototype. CPV-2 infection in puppies was first reported in the early 1980s in South Korea. Since then, a number of dogs infected with CPV-2 that died naturally showed hemorrhagic diarrhea (4). Recent studies have shown that three subtypes of CPV-2 (CPV-2a, CPV-2b, CPV-2a variant) have been identified in fecal samples according to differences of the nucleotide sequence of the VP2 gene, and CPV-2a has been confirmed to be the predominant type in Korean puppies (2, 5). Additionally, the CPV-2a type shows the most severe clinical symptoms (6).

Several techniques such as serum neutralization (SN), hemagglutination inhibition (HI), and indirect fluorescent assay (IFA) tests, enzyme linked immunosorbent assay (ELISA), and one-step immunochromatography assays have been used to detect CPV antibodies in puppies (7, 8). The SN test requires many laboratory facilities and a skilled technician. The IFA test shows a poor correlation with

Received: February 8, 2010/ Revised: March 16, 2010
Accepted: March 18, 2010
*Corresponding author: Dong-Kun Yang. National Veterinary Research and Quarantine Service, 175 Jungang-ro, Anyang-si, Gyeonggi-do 430-757, Korea.
Phone: +82-31-467-1783, Fax: +82-31-467-1797
e-mail: yangdk@nvrqs.go.kr
**This work was supported financially by a grant from the National Veterinary Research and Quarantine Service, Ministry for Food, Agriculture, Forestry and Fisheries, Korea.

immunity (7). The ELISA and one-step immunochromatography kits provide rapid results, but are expensive for large numbers of samples and give an only positive or negative result. Thus, although HI assay cannot differentiate antibodies formed by CPV genotypes, the HI test has been considered as the standard method for detecting CPV antibodies.

Although the CPV outbreak in dogs has been reported in several countries around the world (9, 10), no epidemiological sero-surveillance on CPV-2a has yet been properly conducted in Korea. Serological surveillance of canine diseases would provide information about immune status, and it is also considered important for the development of new vaccines and establishment of vaccination programs to prevent the transmission of CPV infection. We have previously reported the seroprevalence of canine parainfluenza virus 2 (CPIV-2) in stray dogs and the isolation of the CPV-2a strain from a dog showing typical symptoms of CPV infection (11, 12). In this study, sera collected from stray dogs of rescue center between 2006 and 2007 were screened for the presence of antibodies against CPV-2a by HI tests.

MATERIALS AND METHODS

Collection of sera

For the seroprevalence survey, blood samples were obtained from a total of 405 stray dogs that were being housed in 18 rescue centers in South Korea between 2006 and 2007. The blood collection was conducted at 3 weeks after the stray dogs came into rescue center and the blood was taken from cephalic vein. Clotted blood samples were centrifuged (3,000 × g, 10 min), and sera were stored at -20℃ until use. Estimated age was determined according to the dental eruption and dental loss (13).

Virus and cells

CPV-2a strain, KV0901 used in this study was isolated in South Korea in 2009 from feces of naturally infected puppies (11). The CPV-2a had been passaged five times in A72 cells derived from canine fibroblast cells. For the propagation of CPV-2a, monolayered A72 cells were rinsed twice with PBS (pH 7.2), then inoculated with the KV0901 strain and incubated in 5% CO_2 incubator for 5 days. After three freeze-thaw cycles, the harvested virus was clarified by centrifugation (3,000 × g, 15 min) to remove cell debris. The titer of CPV-2a was confirmed by an indirect fluorescent assay using an anti CPV-2 monoclonal antibody and by a hemagglutination assay (HA) using pig erythrocytes (0.6%). The CPV-2a was used as the antigen in the HI test.

Hemagglutination assay (HA) and HI test

The HA test was carried out by preparing serial two-fold dilutions of CPV-2a in 50 μl of Sorensen buffer (pH 6.8) and with 50 μl of 0.6% pig erythrocytes. The HA titer was expressed as the reciprocal of the highest dilution of CPV-2a showing hemagglutination.

The HI test was performed in 96-well microplates as described previously (14), with slight modifications. Briefly, to remove non-specific inhibitors, 50 μl of serum was mixed with 200 μl of 25% kaolin and incubated for 30 min. After pipetting, the kaolin was removed by centrifugation (10,000 × g, 15 min). The clear supernatant was mixed with 5 μl of packed pig erythrocytes to remove any natural agglutinins. After incubation for 1 h at 37℃, the treated serum was separated from the erythrocytes by centrifugation. For the HI test, 4~8 HA units of CPV-2a (in 25 μl) were added to 25 μl of the treated serum. After incubation for 1 h at room temperature, 50 μl of 0.6% pig erythrocytes was added, and the microplates were incubated at 4℃ for 1 h. The HI titer was expressed as the reciprocal of the highest dilution of serum showing complete inhibition of hemagglutination. Serum samples showing HI titers ≥1:10 were considered positive.

Statistical analysis

The chi-square test was used to analyze differences in seroprevalence by age, gender, and region. A p-value of less than 0.05 was deemed to indicate statistical significance.

RESULTS

The results of the seroprevalence of CPV-2a are shown in Tables 1, 2, and Figure 1. The overall prevalence of antibodies against CPV-2a was 93.8% in the 405 serum samples tested. The regional distribution of the seroprevalence of a positive HI titer ranged from 88.3% to 100.0%. Compared with other area, Jeju province showed the highest seropositive rate of 100%. However, there was no significant difference ($\chi^2 = 4.49$; $p = 0.482$) in the regional distribution.

Table 1. Regional distribution of CPV-2a antibodies from the Korean stray dogs

Designation	Province[a]						Total
	GG	GW	CC	JR	GS	JJ	
No. rescue center	4	1	3	3	5	2	18
No. stray dogs sampled	244	14	41	38	60	8	405
No. positive	232	13	38	36	53	8	380
Sero-positive rate[b] (%)	95.1	92.9	92.7	94.7	88.3	100	93.8

[a] GG; Gyeonggi, GW; Gangwon, CC; Chungcheong, JR; Jeonra, GS; Gyeungsang, JJ; Jeju.
[b] $\chi^2 = 4.49$, $p = 0.482$.

Table 2. Distribution of HI antibody titer against CPV-2a according to the age and gender of the Korean stray dogs

Designation	Age				Gender		Total
	< 0.6 m[a]	0.7~2.0	2.1~5.0	> 5.1	Male	Female	
No. stray dog	56	174	108	42	176	204	380
No. positive	62	187	113	43	188	217	405
Sero-positive rate[b] (%)	90.3	93.0	95.6	97.7	93.6	94.0	93.8

[a] m: month. [b] $\chi^2 = 3.21$, $p = 0.361$ for age; $\chi^2 = 0.02$, $p > 0.500$ for gender.

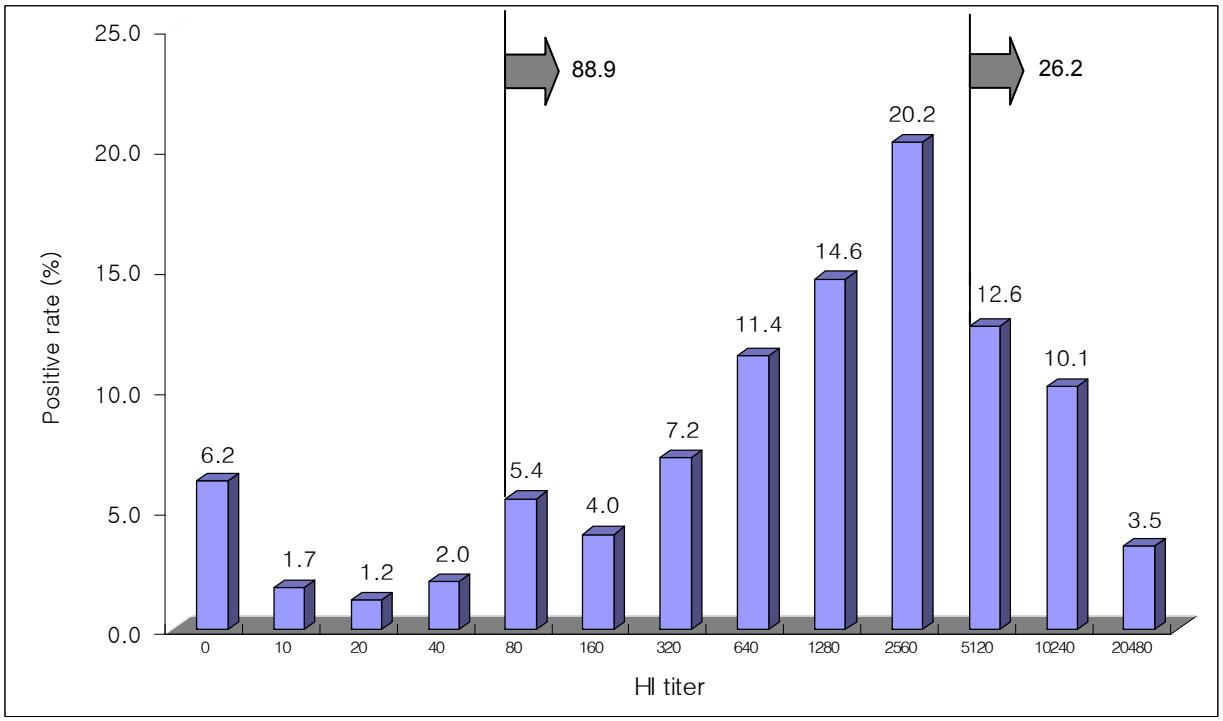

Figure 1. Frequency of distribution of HI titer against canine parvovirus type 2a in 405 Korean stray dogs tested. Titers less than 1:10 were considered negative.

In detail, the regional prevalences were 100% (8/8) in Jeju, 95.1% (232/244) in Gyeonggi, 94.7% (36/38) in Jeonra, 92.9% (13/14) in Gangwon, 92.7% (38/41) in Chungcheong, and 88.3% (53/60) in Gyeungsang provinces. We found no significant difference (χ^2 = 0.02; p > 0.500) in the seropositive rate between male (93.6%) and female (94.0%) dogs. Analysis of the distribution of HI titer against CPV-2a according to the age of the stray dogs showed the highest seropositive rate in dogs older than 5 years; although the linear increase of seroprevalence was shown to age groups, it was not statistically significant (χ^2 = 3.21; p = 0.361). The incidence of the stray dogs showing an HI antibody titer above 1:80 and 1:5120 was 88.9% and 26.2% respectively. Of the strays that had HI antibody titer of 1:10 or more, the most frequent HI titer was 1:2,560 (20.2%).

DISCUSSION

Since the original CPV-2 disappeared around 1984, many countries have reported the occurrence of new antigenic variants, such as CPV-2a/b/c, based on nucleotide analyses of the VP2 gene (7). It has been reported that CPV-2a is the predominant type circulating in Korean puppies (2, 11, 15). Although many reports have dealt with CPV antigens, few publications have addressed the seroepidemiology of CPV-2a in stray dogs. Thus, we conducted a nationwide survey of the presence of antibodies to CPV-2a in stray dogs.

It has been reported that CPV-2a seroprevalence ranged from 64% to 82.9%, depending on the time and district of survey (16, 17). As shown in Tables 1, 2, the overall prevalence of antibodies against CPV-2a was 93.8%. Because CPV vaccines, including a canine distemper, hepatitis, parainfluenza, parvovirus, leptospira. spp combined vaccine (DHPPL), have been widely used in dogs and HI test can detect cross reaction among CPV genotypes, the seroprevalence results for CPV-2a in this study may not represent the true prevalence of CPV-2a wild infection. This positive rate may be higher than those of other countries. At this stage, it is not clear why Korean stray dogs showed such a high seroprevalence rather than those seen in other countries. However, a few suggestions could be made. Firstly, in total, 42 different vaccines associated with CPV have been licensed since the early 1980s. Of those, 34 are live-attenuated vaccines, and 8 are inactivated vaccines; they have been used for the prevention of wild CPV infection in Korean pets (18). Most of the puppies raised in families have been inoculated with single or multivalent vaccines containing CPV antigen(s), according to the vaccine program. Because stray dogs were raised as pet before being abandoned, most stray dogs may show seropositive reactions against CPV-2a. Secondly, the stray dogs in rescue shelters have often been housed together in confined spaces and unsanitary conditions. Thus, transmission of CPV-2a can readily occur. The serological data showed that the regional seroprevalence ranged from 88.3% to 100%, depending on the geographical locations, with no statistically significant differences among regions.

Previous studies reported that dogs with HI titers >1:80 were protected against oral CPV challenge (19, 20). As shown in Figure 1, 89.0% of the stray dogs examined were found to have HI antibody titers of 1:80 or above, indicating that most of the stray dogs were protected against wild CPV-2a infection. Additionally, the HI titer of dogs challenged with wild CPV-2b increased with value of geometric mean 3,043 at day post inoculation 14 (14, 21). In our study, 26.2% of the stray dogs had HI titers higher than 1:5,120, considering that the presence of high HI antibody against CPV-2a may show the evidence of the circulation of CPV-2a in stray dogs.

In conclusion, as stray dogs can play an important role as a reservoir of infectious agent such as canine distemper virus, it is imperative that the management of stray dogs is justified to prevent the transmission of canine infectious diseases.

REFERENCES

1) Appel MJ, Cooper BJ, Greisen H, Scott F, Carmichael LE. Canine viral enteritis. I. Status report on corona- and parvo-like viral enteritides. Cornell Vet 1979;69: 123-33.
2) Kang BK, Song DS, Lee CS, Jung KI, Park SJ, Kim

EM, et al. Prevalence and genetic characterization of canine parvoviruses in Korea. Virus Genes 2008;36: 127-33.
3) Truyen U, Agbandje M, Parrish CR. Characterization of the feline host range and a specific epitope of feline panleukopenia virus. Virology 1994;200:494-503.
4) Han HR, Hwang EK, Rhee YO, Yoo GY. Occurrence of acute viral enteritis in dogs in Korea. Korea J Vet Res 1982;22:167-70.
5) Jeoung SY, Ahn SJ, Kim D. Genetic analysis of VP2 gene of canine parvovirus isolates in Korea. J Vet Med Sci 2008;70:719-22.
6) Moon HS, Lee SA, Lee SG, Choi R, Jeoung SY, Kim D, et al. Comparison of the pathogenicity in three different Korean canine parvovirus 2 (CPV-2) isolates. Vet Microbiol 2008;131:47-56.
7) Carmichael LE. An annotated historical account of canine parvovirus. J Vet Med B Infect Dis Vet Public Health 2005;52:303-11.
8) Oh JS, Ha GW, Cho YS, Kim MJ, An DJ, Hwang KK, et al. One-step immunochromatography assay kit for detecting antibodies to canine parvovirus. Clin Vaccine Immunol 2006;13:520-4.
9) Lamm CG, Rezabek GB. Parvovirus infection in domestic companion animals. Vet Clin North Am Small Anim Pract 2008;38:837-50.
10) Santos N, Almendra C, Tavares L. Serologic survey for canine distemper virus and canine parvovirus in free-ranging wild carnivores from Portugal. J Wildl Dis 2009;45:221-6.
11) Yang DK, Kim BH, Kim YH, Lee KW, Choi SS, Son SW. Genetic analysis of canine parvovirus vaccine strains in Korea. Korean J Vet Res 2009;49:243-8.
12) Yang DK, Yoon SS, Kim BH, Byun JW, Lee KW, Kim YH, et al. Incidence of canine parainfluenza virus 2 in the Korean stray dogs by immunohistochemistry and serology. Kor J Vet Publ Hlth 2009;33:1-12.
13) Dyce KM, Sack WO, Wensing CJG. Textbook of veterinary anatomy. 3rd ed. USA: Saunders, 2002.
14) Carmichael LE, Joubert JC, Pollock RV. Hemagglutination by canine parvovirus: serologic studies and diagnostic applications. Am J Vet Res 1980; 41:784-91.
15) Kim D. Typing and pathogenicity investigation of canine parvovirus isolates from Korea. Extramural research project report NVRQS. pp. 41-69, Anyang, Korea, 2007.
16) Choi DY, Lyoo YS, Kwon HC, Kim YH, Kim DH. Incidence of canine parvo virus infection in Korea, Res Rept RDA 1986;28:108-14.
17) Corrain R, Di Francesco A, Bolognini M, Ciucci P, Baldelli R, Guberti V. Serosurvey for CPV-2, distemper virus, ehrlichiosis and leishmaniosis in free-ranging dogs in Italy. Vet Rec 2007;160:91-2.
18) Korea animal health products association. Import and sales amount of animal health products. pp. 285-312, Seoul, Korea, 2008.
19) Buonavoglia C, Compagnucci M, Orfei Z. Dog response to plaque variant of canine parvovirus. Zentralbl Veterinarmed B 1983;30:526-31.
20) Pollock RV, Carmichael LE. Dog response to inactivated canine parvovirus and feline panleukopenia virus vaccines. Cornell Vet 1982;72:16-35.
21) Elia G, Cavalli A, Cirone F, Lorusso E, Camero M, Buonavoglia D, et al. Antibody levels and protection to canine parvovirus type 2. J Vet Med B Infect Dis Vet Public Health 2005;52:320-2.