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# Incidence of canine viral diseases and prevalence of virus neutralization antibodies of canine distemper virus, adenovirus type 2, parvovirus, and parainfluenza virus type 5 in Korean dogs

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## Abstract

Canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus (CPV), and canine parainfluenza virus 5 (CPIV-5) are the major viral pathogens in dogs. Despite the availability of vaccines for dogs against these 4 viral pathogens, investigations of antibodies against these pathogens have rarely been reported in South Korea. In this study, we investigated the recent incidence of viral diseases in dogs and conducted sero-surveillance for CDV, CAV-2, CPV, and CPIV-5 in Korean dogs. The most frequently diagnosed canine viral disease in Korean dog samples from 2000 to 2022 was CPV infection, which accounted for 48.7% (464/953) of the cases. A total of 400 dog serum samples collected between 2019 and 2022 were screened for the presence of virus-neutralizing antibodies against CDV, CAV-2, CPV, and CPIV-5. The overall seropositivity rates for CDV, CAV-2, CPV, and CPIV-5 were 83.8%, 77.8%, 99.3%, and 82.0%, respectively. The protection rate against CPV was the highest (98.3%) and that against CAV-2 was the lowest (44.8%) in dog sera. Male and female dogs showed no significant differences in seropositivity rates. CDV and CPIV-5 seropositivity increased with age in dogs, and the highest incidence and seropositivity rates of CPV indicated that Korean dogs have been continuously exposed to wild CPV, and that CPV is a pathogen that urgently requires attention among canine viral diseases.

**Keywords:** distemper; parvovirus; adenoviruses; canine; serologic tests

## Introduction

Canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus (CPV), and canine parainfluenza virus 5 (CPIV-5) are the core viral infectious pathogens in dogs worldwide [1]. CDV (genus *Morbilivirus*, family *Paramyxoviridae*) consists of an approximately 15-kb genome and infects the respiratory system, digestive system, brain, and spinal cord, impairing their functions. CDV is transmitted through aerosols from recently infected dogs via the oronasal route [2]. Most dogs infected with CDV develop fever, nasal discharge, conjunctivitis, keratitis, anorexia, and neurological symptoms and may die. On the basis of an analysis of the CDV H gene, CDV has been classified into several gen-

otypes: America-1 and America-2, Asia-1 and Asia-2, Europe, Europe wildlife, Arctic-like, and Africa [3]. In South Korea, CDV belonging to the Asia-1 and Asia-2 genotypes has been reported in dogs and raccoon dogs [4,5].

To date, 2 canine adenovirus serotypes have been identified in dogs. CAV-2 (genus *Mastadenovirus*, family *Adenoviridae*) contains a 35-kb double-stranded DNA genome that encodes 30 open reading frames. Unlike canine adenovirus type 1 (CAV-1), which causes infectious hepatitis, CAV-2 infects the respiratory tissues of dogs and wild carnivores and causes infectious rhinotracheitis [6]. Dogs infected with CAV-2 develop fever, cough, and runny nose, and simultaneous infection with CAV-2 and *Bordetella bronchiseptica* results in more severe respiratory symptoms [7]. CAV-2 infections have been recently reported in dogs and wild raccoon dogs in South Korea [8,9].

CPV (genus *Protoparvovirus*, family *Parvoviridae*) also shows 2 serotypes: CPV-1 and CPV-2. CPV-2 was identified in the late 1970s when a dog was infected with mutated feline panleukopenia virus (FPV). CPV-2 is highly related to FPV, with 98% or more homology [10]. New antigenic variants named CPV-2a, 2b, and 2c have emerged and have replaced CPV-2 worldwide [11]. CPV is highly stable in the environment and infects puppies that have lost maternal antibodies. Puppies infected with CPV develop high fever, anorexia, vomiting, and hemorrhagic diarrhea, and more than 90% of the untreated infected puppies die [12]. The CPV VP2 protein, which constitutes approximately 90% of the viral capsid, is involved in the development of neutralizing antibodies and is used as an antigen to detect CPV antibodies after natural infections [13].

CPIV-5 (genus *Rubulavirus*, family *Paramyxoviridae*), previously known as simian virus 5, is a common pathogen that causes kennel cough in dogs [14]. Parainfluenza virus (PIV) types 1–4 are related to humans and are likely to cause respiratory illnesses in children under 5 years of age, adults over 65 years of age, and people with weakened immune systems [15]. Because PIV-5 has been reported in many animal species, including pigs, cattle, dogs, hamsters, and wild animals, the virus isolated from dogs with respiratory signs is referred to as CPIV-5 in the veterinary field. The genetic homology of CPIV-5 isolated from various animals, including dogs, pigs and cattle, has been reported to be over 99% [16].

Several serological methods, such as virus neutralization (VN), hemagglutination inhibition (HI), indirect enzyme-linked immunosorbent assays, and hemadsorption inhibition tests, have been used to measure antibodies against CDV, CAV-2, CPV, and CPIV-5 [1,17]. Among these serological methods, the VN test, a standard diagnostic method, has been used to accurately mea-

sure the number of antibodies against the 4 pathogens. Although canine viral infections have been reported in South Korean dogs [4,5,8,9], recent incidence data and serologic investigations of major dog pathogens are lacking. Thus, sero-surveillance of these 4 viral pathogens can provide useful information on the immune status of dog populations. Therefore, in this study, we collected and analyzed the incidence of canine viral diseases diagnosed between 2000 and 2022. In addition, serological investigations of CDV, CAV-2, CPV, and CPIV-5 isolated in South Korea were performed using sera from 400 dogs collected between 2019 and 2022.

## Materials and Methods

### Cells and viruses

Vero (ATCC, CCL-81), MDCK (ATCC, CCL-34), and A72 (ATCC, CRL-1541) cells were grown in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum and an antibiotic (penicillin and streptomycin)-antimycotic (amphotericin B) solution (Gibco, USA). Vero, MDCK, and A72 cells were used to measure the levels of antibodies against CDV, CAV-2, CPV, and CPIV-5. CD1901, APQA1701, CPV0901, and QIA-B1201 strains were isolated from naturally infected individuals and used for VN tests for CDV, CAV-2, CPV, and CPIV-5 [8,18–20].

### Collection of data and serum samples

Data on all dog viral diseases reported to the Korea Animal Health Integrated System (KAHIS, www.kahis.go.kr) between 2000 and 2022 were collected and analyzed. In total, 400 serum samples were obtained for the sero-surveillance study of dogs residing in Seoul and Gyeonggi provinces of South Korea from 2019 to 2022. These sera were tested for rabies before traveling abroad and were stored below -20°C until use. For each dog, key information such as sex, age, and breed was collected from the dog owners. All dogs received 2 doses of the rabies vaccine 4 weeks later. No further information was available regarding whether the dogs were inoculated with other vaccines, including the dog core vaccine.

### VN test

VN tests for CDV, CAV-2, CPV, and CPIV-5 were performed in 96-well plates using dog sera inactivated at 56°C for 30 min. Although the cells and viral strains used in the VN tests were different, the experimental procedures for all VN tests were similar. The VN test was performed according to a previously reported method [21]. The medium used for the VN test

against CAV-2 was  $\alpha$ -MEM (Gibco), which was suitable for observing the cytopathic effects (CPE) caused by the APQA-1701 strain. Each well was examined under a microscope to detect virus-specific CPE. The virus neutralizing antibody (VNA) titers were expressed as the reciprocal of the highest serum dilution factor that completely inhibits the CPEs. A VNA titer of 1:2 or higher was considered to indicate positivity against CDV, CAV-2, CPV, and CPIV-5. Thresholds for protective VNA titers were set at 1:32 or higher against CDV and CPIV-5, 1:16 against CAV-2, and 1:64 against CPV [1].

### Statistical analysis

All statistical analyses were performed using R software ver. 4.3.0 (www.cran.org). Chi-square and Fisher exact tests were used to compare the seroprevalence of the categorical variables. The non-parametric Kruskal-Wallis test was used with  $\log_2$ -transformed data of VNA titers to confirm the normal distribution of data. Statistical significance was set at  $p < 0.05$ .

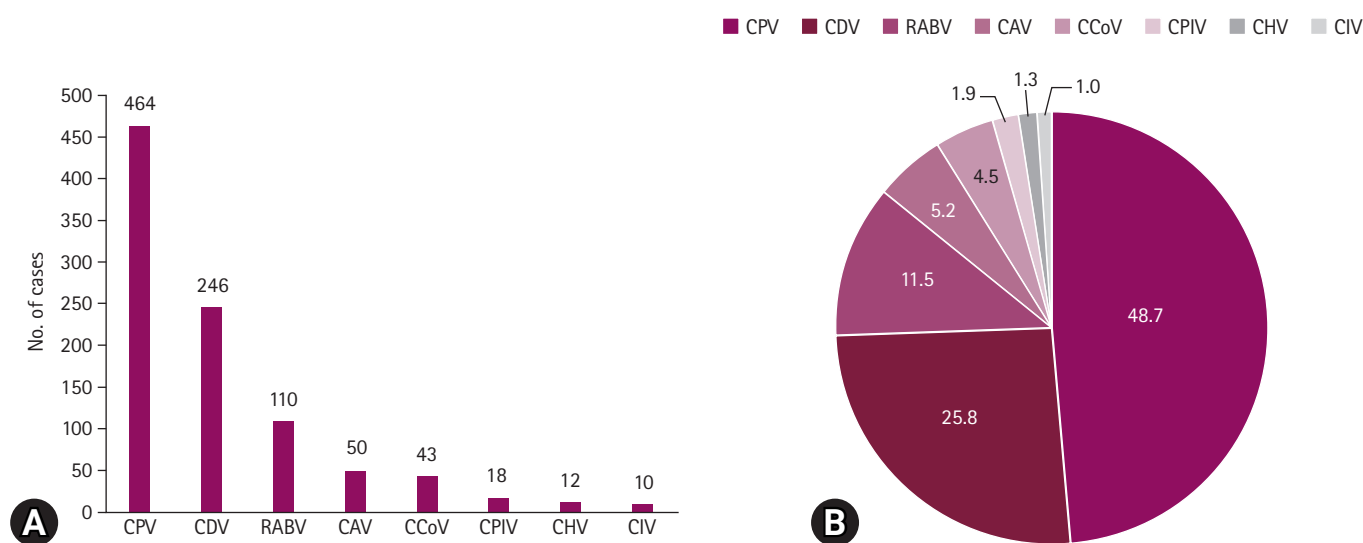
## Results

Canine viral pathogens, including CDV, CAV-2, CPV, and CPIV-5, were investigated between 2000 and 2022 using data from the KAHIS program. As shown in Fig. 1, of the 953 confirmed cases, CPV accounted for the largest number of diagnoses (48.7%, 464/953). The number of CDV, rabies virus (RABV), CAV, canine coronavirus (CCoV), CPIV, canine herpesvirus

(CHV), and canine influenza virus (CIV) infections diagnosed was 246, 110, 50, 43, 18, 12, and 10, respectively. Calciviral infections were not detected.

The seroprevalence rates of CDV, CAV-2, CPV, and CPIV-5 were examined using the VN test in 400 dog serum samples collected from the Seoul and Gyeonggi provinces of South Korea. Of the 400 dogs tested in this study, 213 (53.3%) were male and 187 (46.8%) were female. The mean age of the dogs was 2.1 years. The overall seropositivity rates for CDV, CAV-2, CPV, and CPIV-5 were 83.8% (335/400), 77.8% (311/400), 99.3% (397/400), and 82.0% (328/400), respectively (Table 1). In the analysis of seropositivity rates by year, the range of seropositivity for CPIV-5 varied greatly from 74.0% in 2020 to 90.0% in 2022. The seropositivity rate for CPV showed the smallest difference, ranging from 98.0% in 2020 to 100% in 2019 and 2021. The seropositivity rates for the 4 viruses showed no significant differences in relation to sex. The protection rates against CDV, CAV-2, CPV, and CPIV-5 were 45.3% (181/400), 44.8% (179/400), 98.3% (393/400), and 53.8% (215/400), respectively, and the protection rates against CPV and CPIV-5 increased as dogs aged (Table 2).

Among the 4 age groups, dogs aged > 7 years with CDV and CPIV-5 showed a higher antibody distribution rate than dogs under 0.5 years of age. However, the VNA titers for CPV and CAV-2 showed no significant differences in relation to the age group (Fig. 2). In addition, no significant sex-related differences were found in the VNA titers for the 4 viral pathogens. The



**Fig. 1.** Number of canine viral infections confirmed between 2000 and 2022 in South Korea (A) and percentage of each disease out of the total number of cases diagnosed (953) (B). There have been no reports of rabies since 2014. CPV, canine parvovirus; CDV, canine distemper virus; RABV, rabies virus; CAV, canine adenovirus; CCoV, canine coronavirus; CPIV, canine parainfluenza virus; CHV, canine herpesvirus; CIV, canine influenza virus. All data related to diagnosis were obtained from the KAHIS program (www.kahis.go.kr).

**Table 1.** Seropositivity rates of CDV, CAV-2, CPV, and CPIV-5 using virus neutralization assays in dog blood samples collected from Seoul and Gyeonggi province, South Korea

Year/sex	Seropositivity rates against 4 canine viral pathogens			
	CDV	CAV-2	CPV	CPIV-5
2019	91/100 (91.0)	79/100 (79.0)	100/100 (100.0)	83/100 (83.0)
2020	81/100 (81.0)	70/100 (70.0)	98/100 (98.0)	74/100 (74.0)
2021	81/100 (81.0)	81/100 (81.0)	100/100 (100.0)	81/100 (81.0)
2022	82/100 (82.0)	81/100 (81.0)	99/100 (99.0)	90/100 (90.0)
Male	173/213 (81.2)	169/213 (79.3)	212/213 (99.5)	173/213 (81.2)
Female	162/187 (86.6)	142/187 (75.9)	185/187 (98.9)	155/187 (82.9)
Total	335/400 (83.8)	311/400 (77.8)	397/400 (99.3)	328/400 (82.0)

Values are presented as number positive/number tested (% positive).

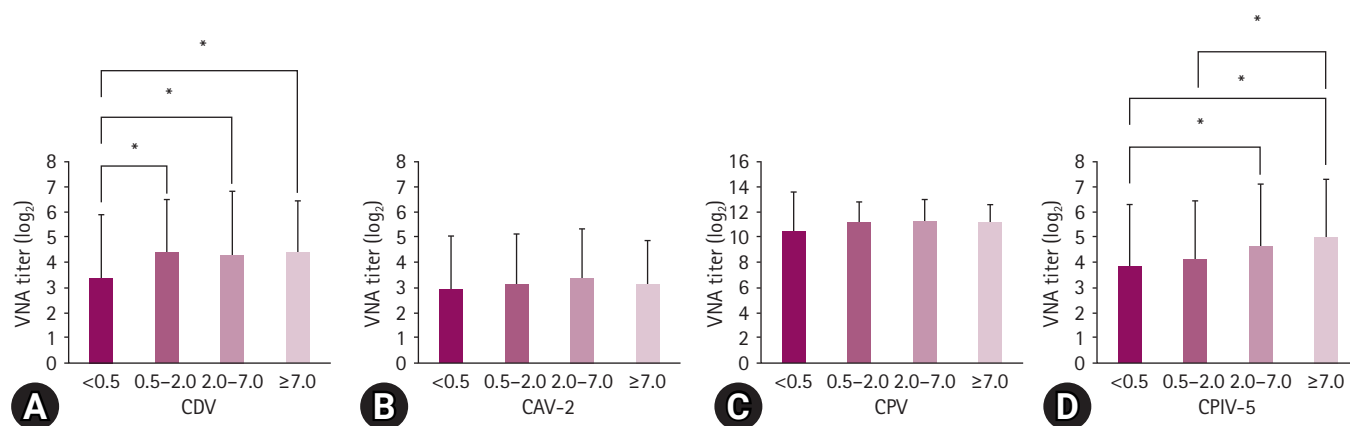
CDV, canine distemper virus; CAV-2, canine adenovirus type 2; CPV, canine parvovirus; CPIV-5, canine parainfluenza virus type 5.

**Table 2.** Protective rates of CDV, CAV-2, CPV and CPIV-5 based on dog age

	Protective rate (%)	p-value	CPV or CPIV-5	Protective rate (%)	p-value
CDV			Total	393/400 (98.3)	
Total	181/400 (45.3)		< 0.5	46/50 (92.0)	< 0.01*
< 0.5	18/50 (36.0)	0.40	0.5-2.0	127/130 (97.7)	
0.5-2.0	65/130 (50.0)		2.0-7.0	157/157 (100)	
2.0-7.0	70/157 (44.6)		≥ 7	63/63 (100)	
≥ 7	28/63 (44.4)				
CAV-2			Total	215/400 (53.8)	
Total	179/400 (44.8)		< 0.5	19/50 (38.0)	< 0.05
< 0.5	20/50 (40.0)	0.51	0.5-2.0	63/130 (48.5)	
0.5-2.0	57/130 (43.9)		2.0-7.0	92/157 (60.5)	
2.0-7.0	77/157 (49.0)		≥ 7	41/63 (65.1)	
≥ 7	25/63 (39.7)				

CDV, canine distemper virus; CAV-2, canine adenovirus type 2; CPV, canine parvovirus; CPIV-5, canine parainfluenza virus type 5.

\*Fisher exact test.



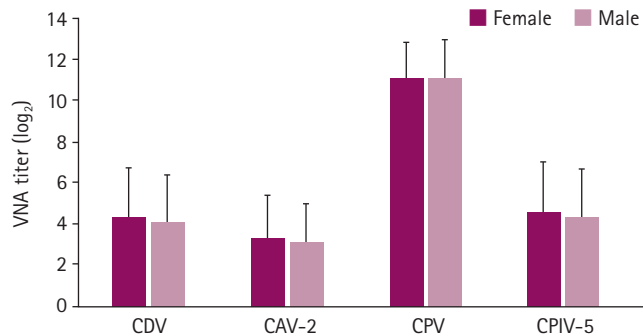
**Fig. 2.** Comparison of virus neutralization antibody (VNA) titers against (A) canine distemper virus (CDV), (B) canine adenovirus type 2 (CAV-2), (C) canine parvovirus (CPV), and (D) canine parainfluenza virus 5 (CPIV-5) in relation to the age of the Korean dogs (Kruskal-Wallis test). As the dogs aged, the mean VNA titers of CDV and CPIV-5 increased (\* $p < 0.05$ ).

mean VNA titers of CDV, CAV-2, CPV, and CPIV-5 were 1:17.1 ( $2^{4.1}$ ), 1:8.6 ( $2^{3.1}$ ), 1:2,194 ( $2^{11.1}$ ), and 1:21.1 ( $2^{4.4}$ ), respectively (Fig. 3). The distribution of VNA titers for the 4 viral pathogens was also analyzed. The most frequent VNA titers against CDV and CAV-2 were  $2^4$  (16.3% and 18.5%, respectively), whereas those against CPV and CPIV-5 were  $2^{11}$  (24.5%) and  $2^5$  (16.5%),

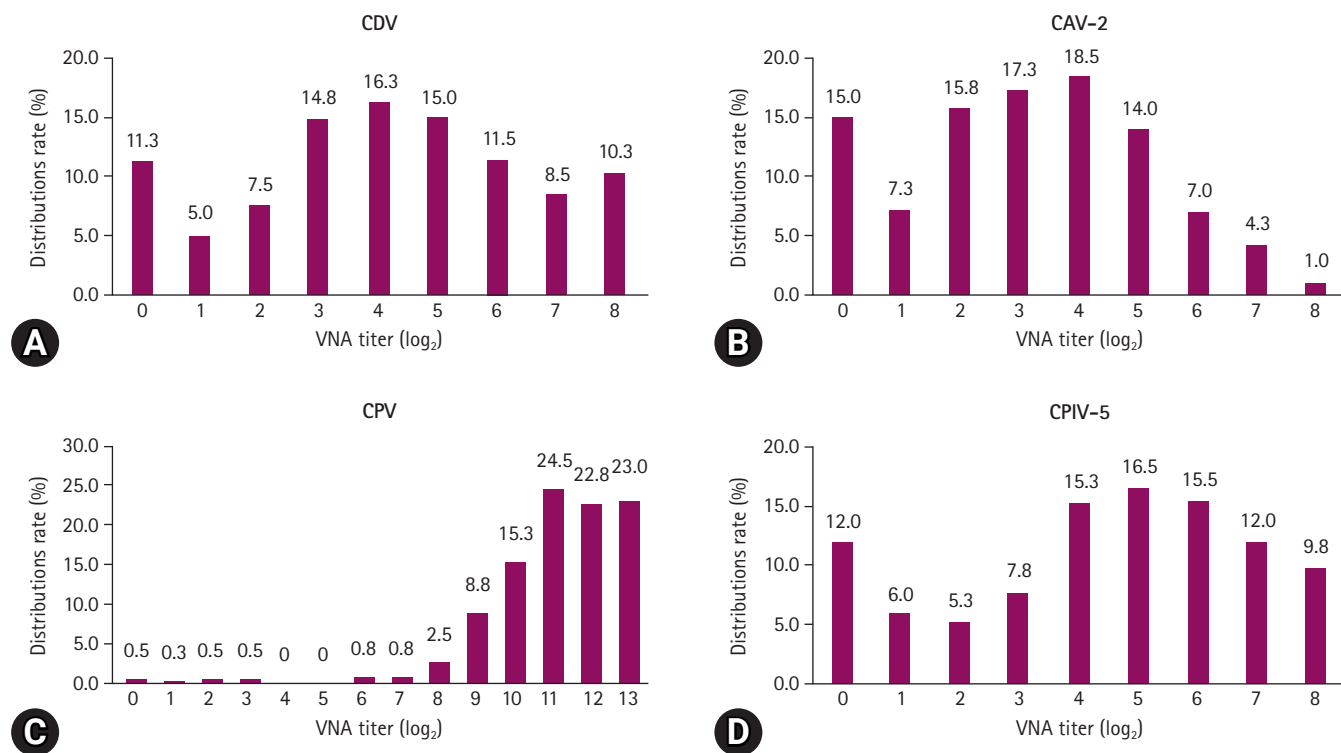
respectively (Fig. 4).

### Discussion

From 2000 to 2022, Korean disease diagnosis agencies diagnosed 953 cases of canine viral diseases based on histopathological findings and molecular biological methods. All diagnostic assessments were performed on dead dogs. In our investigation, 48.7% (464/953) of the dogs diagnosed in Korea were confirmed to have CPV infections, indicating that CPV is the most lethal and frequently diagnosed pathogen that causes death in Korean dogs. Second, 25.8% (246/953) of the dogs were diagnosed as having CDV infections, a highly infectious and fatal disease [22]. The third most frequently diagnosed pathogen was rabies (11.5%, 110/953), which has not been reported since 2014 [23]. The 4th most common pathogen identified was CAV (5.2%, 50/953). There are 2 genotypes of CAV in dogs, CAV-1 and CAV-2; however, these genotypes were not determined in the diagnosis because the diagnosis was based on histopathological assessments. Nevertheless, molecular diagnosis allows for the distinction between CAV-1 and 2. Therefore, it is necessary to subdivide CAV diagnoses. Infections with CCoV, CPIV-



**Fig. 3.** Comparison of virus neutralization antibody (VNA) titers against canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus (CPV), and canine parainfluenza virus 5 (CPIV-5) in relation to the sex of the Korean dogs (Kruskal-Wallis test). The mean VNA titers of CDV, CAV-2, CPV and CPIV-5 were 1:17.1 ( $2^{4.1}$ ), 1:8.6 ( $2^{3.1}$ ), 1:2,194 ( $2^{11.1}$ ), and 1:21.1 ( $2^{4.4}$ ), respectively.



**Fig. 4.** Distributions of virus neutralization antibody (VNA) titers against (A) canine distemper virus (CDV), (B) canine adenovirus type 2 (CAV-2), (C) canine parvovirus (CPV), and (D) canine parainfluenza virus 5 (CPIV-5) in 400 dog serum samples. An VNA titer of 1:2 or higher was considered to indicate seropositivity against the 4 canine viral pathogens.

5, CHV, and CIV in dogs have also been diagnosed at a low frequency (< 5%). Among the diagnosed viral pathogens, CPV and CDV accounted for 74.5% (710/935) of the diagnoses, indicating that CPV and CDV are the major viral pathogens causing death in dogs, and new preventive measures, including vaccines, are required.

The measurement of VNA titers against core canine pathogens can characterize the dog's immune status and the immune response following vaccination [1]. Analysis of VNA titers can also guide preventive measures such as booster vaccination. Most Korean veterinary clinics recommend that dogs begin receiving the distemper, adenovirus, parvovirus, and parainfluenza virus-5 combined vaccine (DAPP) (CDV, CAV, CPV, and CPIV-5 combined vaccine) vaccine at 6 to 8 weeks of age and receive the vaccine 5 times at 2-week intervals in accordance with the guidelines for vaccination of dogs in Korean animal hospitals [24]. Depending on the company, it may be used as the name of the Distemper, Hepatitis virus, Parvovirus and Parainfluenzavirus-5 combined vaccine (DHPP) (CDV, infectious canine hepatitis virus, CPV, and CPIV-5 combined vaccine). Since most dogs included in this study were over 3 months old, most dogs would have received 2 or more doses of the DAPP vaccine.

The CDV used to measure the VNA titer was the CD1901 strain, which was isolated in 2019 and belongs to the Asia-1 genotype [18]. However, the CDV genotype included in the DAPP vaccine is America-1, which shows approximately 92% genetic homology to the CD1901 strain. To predict the protection rate against circulating CDVs in Korea, VNA titers were measured using the CD1901 strain. Protection rates against CDV have been reported to range from 68.6% to 96.0% [1,17]. In this study, the total protection rate against CDV was 45.3%, which is significantly lower than that reported previously. The highest protection rate for dogs by age was less than 50%, indicating the need for booster vaccinations or the development of a new vaccine using the Asia-1 genotype.

CAV-2 seropositivity rates have been reported in many countries, but these rates depend on the age and vaccination status of the dogs [25]. In our study, VNA titers against CAV-2 were measured using the APQA1701 strain, which was isolated from Korean dogs [8], because most DAPP vaccines contain the CAV-2 strain. The seropositivity and protection rates for CAV-2 were 77.8% and 44.8%, respectively. The seropositivity and protection rates were the lowest among the 4 pathogens tested. Therefore, booster vaccinations are recommended to increase the protection rate against CAV-2.

In this study, CPV was the most frequently diagnosed viral

pathogen, and the seropositivity rate against CPV was also the highest at 99.3%. High CPV seropositivity and protection rates indicate that Korean dogs are continuously exposed to wild CPV. CPV-infected dogs can shed the virus into their feces, thereby contaminating the surrounding area. FPV-infected stray cats may also be an additional factor infecting dogs [26]. CPV, which is stable in the environment, can infect vaccinated dogs and increase antibody titers [1]. Dogs with HI titers of 1:80 or higher are known to be protected against a CPV challenge [1,27]. Therefore, we set an VNA titer of  $\geq 1:64$  as the protective antibody titer. Our study showed that 98.3% of the dogs tested had VNA titers  $\geq 1:64$ , indicating that only 1.7% of the dogs are not protected against wild CPV infection and can act as sources of infection. Furthermore, 70.3% of the dogs had VNA titers higher than 1:2,048, assuming that CPV is circulating in the Korean dog population.

CPIV-5 causes problems in the respiratory system through a mixed infection with bacteria, rather than a single infection. Seropositivity rates against CPIV-5 have been reported to range from 28.0% in Sweden to 28.9% in Czechoslovakia [28,29]. Our result showed that 82.0% of dogs had VNA titer of  $\geq 1:2$ , indicating that DAPP vaccination induced a high immune response. Unlike other viral pathogens, the protection rate against CPIV-5 increased from 38.0% to 65.1% with age, indicating that dogs may be continuously exposed to CPIV-5.

In conclusion, canine viral pathogens were investigated in samples digested between 2000 and 2022. The most frequently identified canine viruses by Korean diagnostic agencies are CPV, followed by CDV, RABV, CAV, CCoV, CPIV, CHV, and CIV. The overall protection rate in dogs tested for CDV, CAV-2, CPV, and CPIV-5 was 45.3%, 44.8%, 98.3%, and 53.8%, respectively. In addition, a careful review is needed to determine whether the vaccine currently in use is suitable for the Korean population. Our data on the incidence and sero-surveillance of 4 dog viral pathogens will contribute to the establishment of effective preventive measures for dogs.

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## Data Availability Statement

Contact the corresponding author for data availability.

## Author's Contributions

Conceptualization: Yang DK; Data curation: Cheong YJ, Yang DK; Formal analysis: Yang DK; Funding acquisition: Hyun BH, Yang DK; Investigation: Yang DK; Methodology: Cheong YJ, Hyeon LS, Kim M; Project administration: Yang DK; Software: Yang DK, Kim HH; Validation: Kim HH, Lee HJ, Cheong YJ; Writing—original draft: Yang DK; Writing—review & editing: all authors.

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