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Incidence and Sero-survey of Canine Adenovirus Type 2 in Various Animal Species

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Canine adenovirus type 2 (CAV-2) is the cause of a major respiratory illness in dogs. In this study, we analyzed adenovirus infections in dogs using 2000~2017 data from the Animal and Plant Quarantine Agency (APQA) and conducted a serological survey of CAV-2 infection in six animal species in Korea. In total, 38 of the 3,179 dog samples were confirmed as canine adenovirus infections. In serological survey, 1,028 dog sera, 160 raccoon dog sera, 100 cattle sera, 257 sow sera, 206 horse sera, and 106 cat sera, collected from January 2016 to July 2018, were screened for the presence of anti-CAV-2 antibodies by virus neutralization test. The seropositivity rates for dogs, raccoon dogs, cattle, sows, horses, and cats were 88.5% (910/1,028), 51.3% (82/160), 85.0% (85/100), 48.6% (125/257), 35.0% (72/206), and 2.8% (3/106), respectively. Among dogs and raccoon dogs, 1.9% (20/1,028) and 8.8% (14/160), respectively, had a virus-neutralizing antibody (VNA) titer of over 1:256. A high CAV-2 VNA titer indicates a repeated vaccination or natural infection in Korean dogs and circulation of CAV-2 in raccoon dog populations.

Key Words: Canine adenovirus type 2, Serological survey, Virus neutralization test

No potential conflict of interest relevant to this article was reported.

INTRODUCTION

Most mammalian adenoviruses are causative agents of respiratory, gastrointestinal, and conjunctival diseases in many animal species including human. There are 10 serotypes of adenovirus in cattle, 4 in pigs, 2 in both horses and dogs, and 27 in apes (1). Canine adenoviruses (CAVs) are classified into CAV-1, which causes infectious canine hepatitis, and CAV-2, which induces infectious laryngotracheitis in members of the family Canidae (2, 3). CAV belongs to the genus *Mastadenovirus* of the family Adenoviridae and has a non-enveloped, double-stranded DNA genome of around 32 kb (4, 5). CAV spreads rapidly among non-vaccinated dogs and is transmitted by direct and indirect contact, such as via food bowls, bedding, and respiratory discharge (1).

Diagnosis of CAV infections are usually based on virus isolation by cell culture, histological and pathological findings and molecular biologic results (4). For the serologic survey, virus neutralization (VN), hemagglutination inhibition (HI), indirect fluorescence, and enzyme linked immunosorbent assay (ELISA) tests are used to detect anti-CAV antibodies in animals (6~8). CAV ELISA kits provide rapid results but are expensive and not available for all animal species. To detect anti-CAV antibodies,

the HI test requires human type-O erythrocytes. The VN test is the standard test for CAV antibodies in animals (8).

CAV-2 infections in animals have been reported in several countries (3, 9), including Korea (10~12). Canine combination vaccines including the CAV-2 antigen have been used for the prevention of the CAV-2 infection in dogs since 1987 in Korea. Although CAV-infected animals typically show moderate clinical symptoms, the prevalence of CAV infections among animals in Korea is unclear. CAV-2 was first isolated from a naturally infected Korean dog in 2017. Sero-surveillance of CAV-2 would provide useful information on immunity and prior CAV-2 vaccination in animals. In this study, we analyzed CAV infections in dogs and sera from six animal species were screened for the presence of anti-CAV-2 antibodies.

MATERIALS AND METHODS

Collection of data and sera

Data on CAV infections diagnosed by the Animal and Plant Quarantine Agency (APQA) of Korea from 2000 to 2017 were collected. For sero-surveillance of CAV-2, serum samples were obtained from 1,028 dogs, 160 raccoon dogs, 100 cattle, 257 sows, 206 horses, and 106 cats. Dog, raccoon dog, and cat sera were collected from 2016 to 2018 during development of a rabies vaccine. Bloods were collected from dogs being housed in Gyeonggi, Gyeongbuk, Chungcheongbuk and Gangwon Provinces. Wild raccoon dogs and stray cat bloods were obtained from wildlife rescue centers located in Gangwon and Gyeonggi Provinces. Cattle and sow sera were collected at a slaughterhouse in Gangwon and Gyeongbuk Provinces, respectively in 2017. Horse sera were taken from thoroughbreds by the Korea Racing Authority for the sero-surveillance of seven infectious diseases (West Nile fever, Japanese Encephalitis, equine viral arthritis, vesicular stomatitis, equine infectious anemia, influenza, African horse sickness) in 2017. The clotted blood samples were centrifuged at 3,000 × g for 15 min, and sera were stored at -20℃ until use.

Cells and virus

Vero cells (African green monkey kidney cells, ATCC CCL-1586, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 100 IU/ml penicillin and 10 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA). The CAV-2 strain APQA1701 was isolated from a naturally infected dog in 2017 and had been passaged five times in Vero cells. For the propagation of APQA1701, monolayers of Vero cells in 175 cm^2 tissue-culture flasks were washed twice with phosphate-buffered saline (PBS, pH 7.2), infected with APQA1701, and incubated in a 5% CO_2 incubator for 4 days. After three freeze-thaw cycles, harvested virus was centrifuged at 3,000 × g for 15 min to remove cell debris. The CAV-2 titer was confirmed by the Reed and Muench method and CAV-2 was used in VN tests (13).

Diagnosis of CAV infection

When dead animals were send to APQA from all over Korea for accurate diagnosis, the cause of death was confirmed based on the autopsy, pathological and histological findings, molecular biologic results such as polymerase chain reaction (PCR) and bacteria culture. Diagnoses of CAV infections in dogs were based on the postmortem findings, pathological changes and PCR.

VN tests

VN tests for CAV-2 were carried out in 96-well microplates using Vero cells. A 50 μl aliquot of each dilution of heat-inactivated (56℃ for 30 min) serum was mixed with an equal volume of 200 $TCID_{50}$/0.1 ml of CAV-2 and incubated at 37℃ for 1 h. Vero cells (100 μl in DMEM containing 5% FBS, n = 20,000 cells) were added to each well, the microplates were incubated for 5 days at 37℃ in a 5% CO_2 incubator, and virus-induced cytopathic effect (CPE) was evaluated under a microscope. The VN

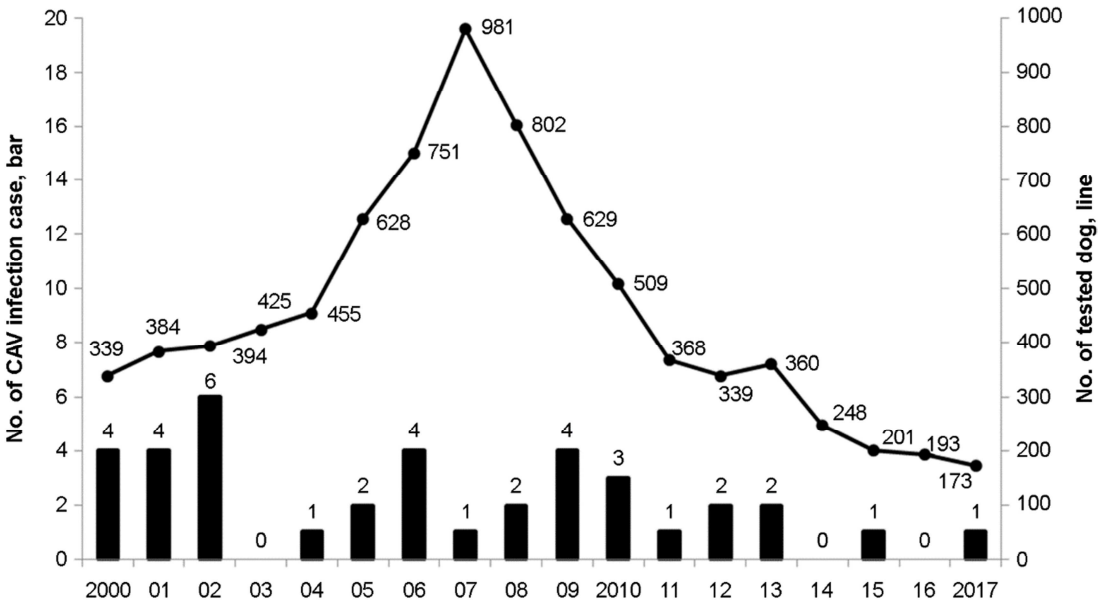

Figure 1. Number of canine adenovirus (CAV) infections confirmed by the Animal and Plant Quarantine Agency (APQA) since 2000 (Y axis on the left) and number of dog samples sent to the APQA for diagnosis (Y axis on the right). Of 8,179 dog samples, 38 had CAV infections based on the autopsy, pathological and histological findings and polymerase chain reaction.

titer was expressed as the reciprocal of the highest serum dilution that completely inhibited the viral CPE. Serum was diluted 1:2 to 1:256; an antibody titer of ≥ 1:2 was considered positive.

RESULTS

From 2000~2017, 38 dogs were confirmed to have CAV infection. Because CAV genotype was not distinguished at diagnosis, it was expressed as CAV infection. Six CAV infections in dogs were diagnosed in 2002; the annual average was 2.11 CAV infections (Fig. 1).

The overall CAV-2 sero-positivity rate was 88.5% (910/1,028) in dogs, 51.3% (82/160) in raccoon dogs, 85.0% (85/100) in cattle, 48.6% (125/257) in sows, 35.0% (72/206) in horses, and 2.8% (3/106) in cats (Fig. 2). As shown in Table 1, 86.6% (910/1,028) of dogs had a CAV-2 antibody titer of 1:128 or less and were considered vaccinated; 1.9% (20/1028) of dogs had a VNA titer of 1:256 and were presumed to have had a repeated vaccination or natural CAV-2 infection. Of the dogs with a VNA titer of 1:2 or more, the most frequent VNA titer was 1:16 (22.1%). Among raccoon dogs, 51.3% (82/160) had a VNA titer of 1:2 or more, and the 23.8% (38/160) with a VNA titer of 1:128 or more were continuously exposed to CAV-2 in their populations. Among cattle, 85% (85/100) had a VNA titer of 1:2 or more; their VNA titers ranged from 1:2 to 1:128. Among sows, 48.6% had VNA titers of 1:2 to 1:32. Although 35.0% of the horses had a VNA titer of 1:2 or more, 94.4% (68/72) had a VNA titer of 1:8 or less. Cats showed the lowest CAV-2 antibody positivity rate of 3.5% (3/106).

DISCUSSION

Since the canine hepatitis virus was first propagated in tissue culture in 1954 (14), CAV-1 and CAV-2 infections of dogs, wolves, and foxes have been reported worldwide (15~17). In Korea, although CAV-1 infection in a Eurasian river otter and fennec fox

Table 1. Distribution of CAV-2 virus neutralization antibody titers in dogs, raccoon dogs, horses, cattle, sows, and cats

Animal species	Dog	Raccoon dog	Horse	Cattle	Cat
VN titer (log_2)	No. of positive (%)	No. of positive (%)	No. of positive (%)	No. of positive (%)	No. of positive (%)
0	118 (11.5)	78 (48.8)	135 (65.2)	15 (15)	103 (97.2)
1	83 (8.1)	11 (6.9)	44 (21.3)	15 (15)	0 (0)
2	148 (14.4)	3 (1.9)	18 (8.7)	11 (11)	0 (0)
3	169 (16.4)	5 (3.1)	6 (2.9)	21 (21)	1 (0.9)
4	227 (22.1)	4 (2.5)	3 (1.4)	16 (16)	0 (0)
5	145 (14.1)	10 (6.3)	1 (0.5)	5 (5)	1 (0.9)
6	78 (7.6)	11 (6.9)	0 (0)	16 (16)	0 (0)
7	40 (3.9)	24 (15.0)	0 (0)	1 (1)	0 (0)
8	20 (1.9)	14 (8.8)	0 (0)	0 (0)	1 (0.9)
Total	1,028	160	207	100	106

Dogs and raccoon dogs known as natural host had higher virus neutralization antibody titer than non-carnivores species.

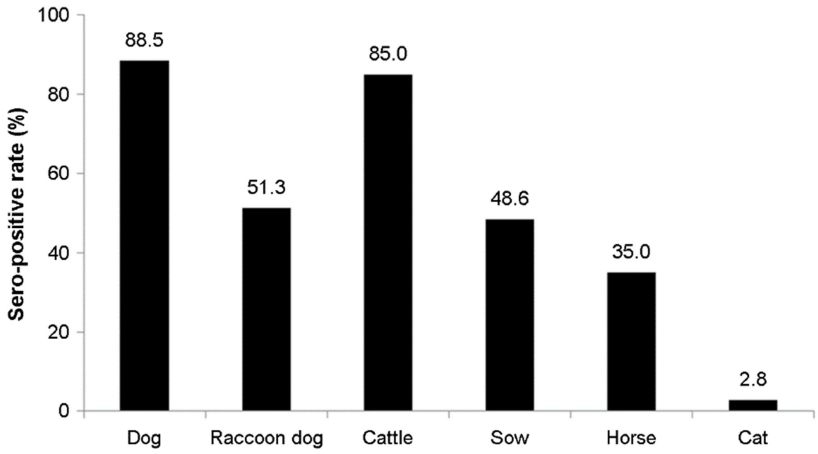

Figure 2. Sero-prevalence of CAV-2 in dogs, raccoon dogs, cattle, sows, horses, and cats. The overall sero-prevalence of CAV-2 was 88.5% (910/1,028) in dogs, 51.3% (82/160) in raccoon dogs, 85.0% (85/100) in cattle, 48.6% (125/257) in sows, 35.0% (72/206) in horses, and 2.8% (3/106) in cats.

have been documented (10, 11), the prevalence and sero-epidemiology of CAV-2 in dog and other animals were unknown. Thus, we collected CAV infection cases from APQA and conducted a serologic survey of CAV infections in dogs, raccoon dogs, cattle, sows, horses, and cats.

Since 2000, 8,179 dog samples have been sent to the APQA of Korea for diagnosis. A total of 38 CAV infection cases in dogs were confirmed by histopathology and PCR during this period. In 2002, six dogs were diagnosed with CAV infection, but there were no CAV infections in 2003, 2014, or 2016. The prevalence of CAV-2 in asymptomatic dogs in US animal shelters was reported as 12.5% (18). The low prevalence of CAV infection in Korea may be due to use of a combination vaccine against

CAV-1 and CAV-2. It is well known that the high antigenic similarity between CAV-1 and CAV-2 results in cross protection (19). Such vaccination induces anti-CAV neutralizing antibodies in almost all dogs (20).

The anti-CAV antibody prevalence in dogs worldwide is 30 to 82% (21). In this study, the overall prevalence of anti-CAV-2 antibodies in dogs was 88.5%. Our higher frequency of anti-CAV-2 antibodies may be because most of the dogs tested were older than 6 months of age and may have received anti-CAV vaccines as puppies. It is also reported that the duration of a strong serological response to the canine core vaccine is > 18 months (20). However, 11.5% of the dogs did not have anti-CAV-2 antibodies, indicating possibility to be sporadic CAV-2 infection in dogs likely to be exposed to CAV.

We also conducted a serological survey of raccoon dogs (suborder *Caniformia*) for CAV-2 infection. The prevalence of CAV-1 or 2 infection in raccoons is 0 to 12% in Japan and the US (22, 23). However, 51.3% of Korean raccoon dogs had VNA against CAV-2 in this study. The difference in the seropositivity rate is presumed to be due to the serotype of CAV-2, which is readily transmitted by respiratory discharge. Furthermore, 23.8% of raccoon dogs had a VNA titer of over 1:128, indicating repeated exposure of the past to CAV-2 (23) and that the virus is circulating among the raccoon dog population. As unintentional contact may be source of CAV infection, unvaccinated dogs should not be permitted to come into contact with wild raccoon dogs.

Ten, four, and two types of adenoviruses are known to infect cattle, sows, and horses, respectively, because they can replicate in a wide range of epithelial cells (24, 25). The prevalence of VNA against CAV-2 was investigated in four farm animal species because many farms have dogs. The prevalence of VNA to CAV-2 in this study was high in cattle (85.0%), moderate in sows (48.6%), and horses (35.0%), but low in cats (2.8%). However, most VNA titers were below 1:8, indicating that CAV-2 has low pathogenicity in non-carnivores.

In conclusion, dogs showed the highest CAV-2 sero-positivity rate among six animal species, indicating that most dogs have received canine core vaccines. Because a small number of dogs were estimated to have recently been exposed to CAV-2, vaccination of dogs is required to prevent CAV-2 respiratory disease. In addition, cattle, sows, horses, and cats showed varying CAV-2 sero-positivity rates. Therefore, further studies should determine whether CAV-2 causes disease in these animals.

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