Detection of Antibody to Canine Distemper Virus in Wild Raccoons (*Procyon lotor*) in Japan

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**ABSTRACT.** Canine distemper virus (CDV) causes a lethal disease among members of the *Carnivora*. To clarify the distribution of CDV in wild animals, we examined 106 raccoon sera collected from two prefectures in Japan, Hyogo and Osaka, from 2005 to 2007. Among them, 34 raccoons (32.1%) possessed a virus-neutralizing (VN) antibody to KDK-1 strain (genotype Asia-1). There was no significant difference in seroprevalence of CDV regardless of places, gender, and body weights. In Hyogo, a geometric mean of VN titers to CDV was significantly higher than that to Onderstepoort (vaccine strain), indicating that KDK-1-like CDV different from vaccine strain might have spread among raccoon population in Hyogo. In conclusion, CDV is epidemic among feral raccoons in Japan, suggesting that CDV might have been spreading among Japanese wild animals.

**KEY WORDS:** canine distemper, Japan, raccoon.

Canine distemper virus (CDV) infects many animal species belonging to the *Carnivora* and causes lethal disease to them. In Serengenti National Park in Tanzania, an epidemic of CDV spread through lions in 1994, resulting that 30% of lions were dead [13]. In some Channel Islands of California, island foxes were infected with CDV and the number rapidly decreased [2], resulting that the four subspecies were listed as federally endangered [15]. In Japan, some wild animals including raccoon dogs (*Nyctereutes procyonoides*) and masked palm civets (*Paguma larvata*) were dead by CDV infection [5, 7, 9, 12, 14].

Raccoons (*Procyon lotor*) are also infected with CDV and the infection is sometimes lethal [6]. In North America, raccoons are thought to be reservoirs of CDV to wild and zoo animals [1]. Raccoons are widely distributed throughout Japan and the number of raccoons has increased. In this study, CDV infection to raccoons was serologically examined.

Vero cells (JCRB Number; JCRB9013) were cultured in Eagle’s minimum essential medium (EMEM; GIBCO, U.S.A.) with 5% heat-inactivated fetal calf serum (FCS; CELlect, MP Biomedicals, U.S.A.), 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO₂. Canine-originated A72/cSLAM and feline-originated CRFK/cSLAM cells expressing canine signaling lymphocyte activation molecule (SLAM) were grown with Dulbecco’s modified Eagle’s medium (DMEM; GIBCO, U.S.A.) supplemented with 10% FCS and antibiotics at 37°C in 5% CO₂ [11]. CDV KDK-1 (genotype Asia-1) [9] and Onderstepoort (a live attenuated vaccine strain) strains were propagated in A72/cSLAM and Vero cells, respectively. Total 106 sera were collected from raccoons captured in 2 prefectures, Hyogo and Osaka, under an official control and eradication program of feral raccoons. Of these, 56 were collected in Hyogo from May 2005 to June 2006 and 50 were collected in Osaka from June 2006 to February 2007. All sera were inactivated by incubation at 56°C for 30 min and then kept at –20°C until use.

Virus-neutralizing (VN) test to KDK-1 [10] was carried out by 75% plaque-reduction neutralizing test (PRNT<sub>75</sub>) using our established cell line, CRFK/cSLAM [11]. For the first screening of CDV-positive raccoons, 10 μl of sera was added to 90 μl of virus solution containing 100 plaque forming unit (PFU) of KDK-1 diluted with DMEM supplemented with 2% FCS and incubated at 37°C for 1 hr (1:10 dilution). Then, 50 μl of the mixture was added to each well of 24 well plate (SUMITOMO BAKELITE, Tokyo, Japan) that were subconfluented with CRFK/cSLAM and the plate was incubated at 37°C for 1 hr, washed twice with DMEM and overlaid with DMEM containing 0.8% agarose and 10% FCS. Plates were then incubated at 37°C in 5% CO₂ for 3–4 days. Cells were fixed with 5% buffered formaldehyde solution for 1 hr and agarose layers were removed. After staining with crystal violet, plaques were counted. Sera that reduced plaques by more than 75% in comparison with the mean number of plaques in control wells were considered to be positive. To analyze the results statistically, chi-square and Fisher’s exact probability tests were performed. The significance level was *P*=0.05.

Thirty-four in 106 raccoons (32.1%) possessed VN anti-
body to CDV (Table 1). Although 19 (33.9%) of 56 raccoons in Hyogo and 15 (30.0%) of 50 in Osaka were seropositive, there was no significant difference in seroprevalence to CDV between regions ($p > 0.05$). In addition, 39.3% of males and 24.0% of females were seropositive for CDV infection, but there were no significant differences by gender ($p > 0.05$). Raccoons are generally thought to be adults when body weights are over approximately 4.0 kg and juveniles when weights are below 4.0 kg [3]. Based on these weights, there were no significant differences in seroprevalence to CDV between adults (36.0%) and juveniles (22.6%) ($p > 0.05$).

Next, in order to determine the VN titers of CDV-positive sera, sera were diluted to 1:10 and then serially two-fold diluted with DMEM containing 2% FCS. Diluted sera were mixed with equal volumes of virus solution containing 100 PFU of KDK-1 or Onderstepoort, followed by incubation at 37°C for 60 min. Mixtures were added to CRFK/cSLAM and PRNT$_{75}$ was carried out. Titer of VN antibody was expressed as the highest dilution of serum that reduced plaques by more than 75% in comparison with control wells without serum.

The geometric means of VN titer were compared between Hyogo and Osaka prefectures (Fig. 1). In Hyogo, the mean of VN titer to KDK-1 was significantly higher than that to Onderstepoort ($p < 0.05$). In Osaka, the VN titers to KDK-1 and Onderstepoort were very similar. When 4-fold higher VN titer is considered to be significant, 10 in 19 raccoons in Hyogo had significantly higher VN titers to KDK-1 than those to Onderstepoort. On the other hand, only one raccoon in Osaka had significantly higher VN titer to Onderstepoort than that to KDK-1. These results indicated that CDV similar to KDK-1 might have infected raccoons in Hyogo.

In Japan, many dogs are inoculated with the live attenuated CDV such as Onderstepoort and the incidence of CD is thought to decrease in dogs. On the other hand, some CDV cases in wild animal populations, raccoon dogs, masked palm civets, badgers (*Meles meles*) and so on, were reported [12, 14, 16]. Our data indicated that raccoons in Japan were also infected with CDV. To our knowledge, this is the first report of the large sero-surveillance of CDV-infection to raccoons in Japan. There is no information of raccoon death by CDV infection in Japan. However, raccoons in North America were dead by CDV infection [6], indicating that CDV spreading among raccoons in Japan might be less virulent to raccoons. On the other hand, there was an epidemic of CDV among raccoon dogs and a weasel in Japan in 2007. In the region, over 50% of raccoons became seropositive for

<table>
<thead>
<tr>
<th>Region</th>
<th>Sex</th>
<th>Body weight</th>
<th>Number of examined samples</th>
<th>Number of CDV-positive samples</th>
<th>Percentage of CDV-positive animals</th>
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<tbody>
<tr>
<td></td>
<td>Hyogo</td>
<td>Osaka</td>
<td>Male</td>
<td>Female</td>
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<td>56</td>
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a: Sera that showed 75% plaque reduction by KDK-1 at 1:10 dilution were judged as positive.
CDV, but death of raccoons was not reported, suggesting that raccoons in Japan might be resistant to CDV infection (Manuscript in preparation). Further CDV surveillance in raccoons will be required to clarify the virulence of CDV to raccoons and the role of raccoons in spreading CDV in Japan.

The recent CDV isolates from dogs were genetically distant from the vaccine strain and belong to two genotypes, Asia-1 and Asia-2 [4, 9]. Our results indicated that CDV similar to genotype Asia-1 infected to raccoons in Hyogo. On the other hand, titer of antibody to KDK-1 was similar to that to Onderstepoort in raccoons in Osaka. In our preliminary data, dogs inoculated with Onderstepoort possessed similar antibody titers to KDK-1 and Onderstepoort. In Osaka, Onderstepoort-like virus might infect raccoons. Now, we are attempting to isolate CDV from raccoons.

In this study, many raccoons were infected with CDV, indicating that CDV has been spreading and there are still high risk of CDV infection to dogs and other carnivores in Japan.

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REFERENCES