

Serological Evidence of Coronavirus Infection in Feral Raccoons in Hokkaido, Japan

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北海道に生息するライグマにおけるコロナウイルス感染

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ABSTRACT. Coronavirus infections in raccoons in Hokkaido, Japan, were identified by serological analysis of 379 serum samples. In the virus neutralization tests, the antibody for transmissible gastroenteritis virus (TGEV), a group I coronavirus, was detected in 11 (3%) serum samples, which were further tested for canine coronavirus (CCoV), and 5 sera showed positive results. The antibody for bovine coronavirus (BCoV), a group II coronavirus, was not detected in any of the serum samples. These results indicate an infection of group I coronaviruses in the feral raccoons in Hokkaido, Japan.

Key words: Coronavirus, Raccoon, Serological survey

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Coronaviruses are divided into three groups according to their serological cross-reactivity and genome organization [1]. To date, groups I and II have been isolated from mammals, and group III has been isolated from birds. In 2002, a previously undetected human coronavirus disease known as severe acute respiratory syndrome (SARS) appeared in China and killed nearly 800 people during the period between November 2002 and July 2003. This emerging virus was detected not only in humans but also in several wild animals [2]. Therefore, it is believed that the host range of the SARS virus evolved from wild-animal hosts to humans. Thus, it is possible that other unidentified coronaviruses are circulating as potential zoonotic agents in animal populations. Since the SARS epidemic, several new coronaviruses have been identified in humans [3, 4], geese, pigeons, and mallards [5].

Raccoons (*Procyon lotor*) are native to and widespread in North America; in the eastern states of the United States,

they are the reservoirs for the raccoon rabies virus. This virus spread along the eastern seaboard to Canada, and it was detected in Ontario in 1999 [6]. Raccoon rabies is a significant threat to domestic animals and humans in both countries. In Japan, since the 1970s, a large number of raccoons have been imported as pets from the United States. In Hokkaido, the northernmost island of Japan, the escape and deliberate release of pet raccoons over the last 20 years has led to the creation of a naturalized population of raccoons, which is centered principally in the southern part of the Ishikari Plain [7]. These wild animals may play a role in the transmission of pathogens to humans and domestic animals, similar to that observed in the case of raccoon rabies in the United States and Canada. In fact, several pathogens, including *Babesia microti*-like parasite [8] and gastrointestinal helminths [9], and antibodies to pathogens such as *Ehrlichia* and *Anaplasma* [10] have been detected in raccoons in Japan. Furthermore, in Colorado, the United States,

electron microscopic studies of the fecal samples obtained from a juvenile raccoon showing dehydration with diarrhea and mucoid oculonasal discharge revealed the presence of coronavirus- and parvovirus-like particles [11]. However, the potential of raccoons as reservoir animals for coronaviruses is still not clearly defined. In the present study, we performed serological analysis to detect coronavirus infection in feral raccoons in Hokkaido, Japan.

Serum samples were collected from 379 raccoons in west-central Hokkaido; these raccoons had been captured for population control directed by the Hokkaido government in 2004. The serological investigations were performed by using virus neutralization (VN) tests for group I and II coronaviruses. We conducted tests for three group I coronaviruses, namely, transmissible gastroenteritis virus (TGEV) strain TO-163, porcine epidemic diarrhea virus (PEDV) strain NK94P6, and canine coronavirus (CCoV) strain 1-71. Briefly, a serially diluted serum sample was reacted in duplicate with hundred 50% tissue culture infective dose (TCID₅₀) of each virus, which was followed by incubation for 1 hr at 37°C. After incubation, the serum-virus solution was transferred onto the monolayered cells (CPK cells for TGEV, Vero-KY7 cells for PEDV, and CRFK cells for CCoV), cultured in microplates, and incubated for 5 days at 37°C. Antibody titers were expressed as the reciprocal of the highest serum dilution that completely inhibited cytopathic effect (CPE). Because of the limited amount of serum, the serum samples were diluted 1:2 for TGEV, 1:8 for PEDV, and 1:4 for CCoV. We detected neutralizing antibodies for TGEV in 11 (3%) of the 379 feral raccoon serum samples, with a titer ranging between 2 and 64; however, we could not detect antibodies for PEDV (Table 1). Within group I

coronaviruses, TGEV, PRCV (porcine respiratory coronavirus), CCoV, FIPV (feline infectious peritonitis virus), and FECV (feline enteric coronavirus) show antigenic relationships on the basis of cross-reactivities observed in the VN test [12, 13]. However, although PEDV has been classified as group I, it does not show cross-reactivity with TGEV in the VN test [14]. We performed a cross-neutralization test between TGEV and PEDV by using antisera raised against each virus. These results confirmed that there was no cross reactivity between these viruses (data not shown). The 11 TGEV-positive serum samples in this study were tested for neutralizing activity with canine coronavirus (CCoV), and 5 of them showed positive results (Table 1). This indicated that the feral raccoons in Hokkaido were infected with coronavirus, which is antigenically related to TGEV and CCoV.

Furthermore, we performed VN tests for a group II coronavirus, bovine coronavirus (BCoV) strain Kakegawa. HRT-18G cell monolayers grown in microplates were inoculated with the serum-virus solution and incubated for 5 days at 37°C. None of the raccoon sera reacted with BCoV (data not shown).

In this study, 11 (3%) of the 379 feral raccoon sera were found to be seropositive for TGEV. However, it is not known whether this seropositivity was because of a known TGEV-related virus infection or because of a novel group I coronavirus. This aspect can be clarified by isolating the virus from the raccoon samples and analyzing the viral genome.

Our findings indicate an infection of group I coronaviruses in the feral raccoons in Hokkaido, Japan. There are no significant epidemiological associations among captured place, estimated age, and sex of raccoons showed seropositive in this study. Continuous monitoring is required to manage the raccoons and prevent zoonosis and transmission of diseases to domestic animals.

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要 約

北海道に生息するアライグマにおけるコロナウイルス感染状況を血清学的解析により調査した。血清 379 検体中、1 型の伝染性胃腸炎ウイルス (TGEV) および犬コロナウイルス (CCoV) に対してそれぞれ 11 および 5 検体が中和抗体陽性を示した。2 型の牛コロナウイルス (BCoV) に対しては全て陰性を示した。これらからアライグマには 1 型コロナウイルスに感染している個体が存在すると考えられた。

キーワード：アライグマ, 抗体調査, コロナウイルス

Table 1. Serum antibody titers of the raccoons for TGEV, PEDV, and CCoV

| Serum No. | Virus | | |
|-----------|------------------|------|------|
| | TGEV | PEDV | CCoV |
| MA12 | 64 ¹⁾ | < 8 | 16 |
| MA45 | 2 | < 8 | < 4 |
| MA52 | 4 | < 8 | < 4 |
| MA54 | 4 | < 8 | 4 |
| MA80 | 2 | < 8 | < 4 |
| MA134 | 32 | < 8 | 8 |
| MA164 | 64 | < 8 | 16 |
| MA179 | 2 | < 8 | < 4 |
| MA190 | 4 | < 8 | < 4 |
| MA195 | 64 | < 8 | 64 |
| MA235 | 2 | < 8 | < 4 |

1) Expressed as the reciprocal of the highest serum dilution that inhibited cytopathic effect (CPE)

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