Short Communication

Molecular Survey of Rickettsial Agents in Feral Raccoons (Procyon lotor) in Hokkaido, Japan

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SUMMARY: Rickettsial infection in feral raccoons (Procyon lotor) in Hokkaido, Japan was analyzed by molecular methods. Genus-specific nested polymerase chain reaction (PCR) analysis based on the Rickettsia citrate synthase (gltA) gene showed that 13 of 699 raccoons (1.9%) examined were positive for Rickettsia. Twelve of the 13 partial gltA sequence amplicons were successfully analyzed. The nucleotide sequence of one amplicon was identical to both Rickettsia heliogiangensis and R. japonica, one was identical to R. felis, and the rest to R. helvetica. This is the first report on the detection of rickettsial agents in peripheral blood of raccoons.

The raccoon is a medium-sized carnivore native to North America; however, a large number of raccoons have been imported from North America as pets since the 1970s (1). Subsequently, large numbers of raccoons have naturalized in many parts of Japan due to the intentional release or escape of pet raccoons (2). Feral raccoons cause heavy damage to crops and native ecosystems in Japan. Furthermore, raccoons may have introduced nonindigenous pathogens into Japan, such as rabies, raccoon ascarid, and rickettsiosis (3), which have been reported in the United States. Because of these problems, the Hokkaido Government initiated a feral raccoon management program in 1999.

Spotted fever group (SFG) Rickettsia is a significant emerging infectious disease whose principal clinical features are fever and rash. In Japan, the first case of rickettsiosis caused by Rickettsia japonica was reported in Tokushima Prefecture in 1984 (4). Other SFG Rickettsia spp. have recently been detected in Japan, including Rickettsia helvetica (5), R. tamurae (6), R. asiatica (7), and R. tarasevichiae (8); however, little epidemiologic data is available (i.e., vectors and reservoir animals). We therefore aimed to characterize rickettsial pathogens by a molecular analysis of peripheral blood samples obtained from feral raccoons in Japan. Another objective of this study was to clarify the epidemiologic role of raccoons for these pathogens in Japan.

A total of 699 raccoons were captured between May and October 2007 and between March and October 2008 as part of raccoon population control programs implemented by the Hokkaido Government and the Ministry of the Environment in west-central Hokkaido, the northernmost of the main islands of Japan. Blood samples were collected from 699 raccoons, and DNA was extracted with a QIAGEN DNA Mini Kit (Qiagen GmbH, Hilden, Germany). DNA samples were stored at −20°C in 200 μl of Tris-EDTA (TE) buffer until further use.

Nested PCR was performed with genus-specific primers for the rickettsial citrate synthase (gltA) gene (9), and the primer pair RpCS.877p and RpCS.1273r was used for the first amplification. The first round of PCR was carried out in a 25-μl reaction mixture (5 μl of DNA template) under the following conditions: 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 90 s. The resulting PCR products were then used as a template for the second amplification with primers RpCS.896f and RpCS.1258n. Cycling conditions for the second round of PCR were the same as the first round, except that annealing was carried out at 56°C for 30 s. DNA extracted from the Rickettsia AT-1 strain was used as a positive control, and distilled water was used for the blank control. PCR products were purified with the Qiaquick PCR purification kit (Qiagen) and sequenced as described previously (9). Sequence homology searches of the PCR products were performed with the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information).

According to genus-specific nested PCR analysis, 13 of 699 (1.9%) raccoons were positive for Rickettsia. In 12 samples, approximately 322 bp of the gltA gene, excluding the primer region, was successfully sequenced. These sequences have been deposited in GenBank under accession numbers HM049647 to HM049658. One amplicon showed 100% nucleotide identity with both R. japonica (AY743327) and R. heilongjiangensis (AY285776), and another was identical to R. felis (U33922). The remaining amplicons were identical to the gltA gene of R. helvetica (AM418450).

To distinguish between R. japonica and R. heilongjiangensis, species-specific PCR for the R. japonica 17-kDa antigen gene was performed with the primers Rj5 and Rj10 (10). Given the negative result, a conclu-
sive determination of whether the amplicons were sequences of *R. japonica* or *R. heilongjiangensis* could not be made. Because sequences of *R. heilongjiangensis* and *R. japonica* are very similar (11), other gene sequences will need to be examined in order to determine which species the samples belong to. The introduction of *R. heilongjiangensis* into Hokkaido from Russia is a possibility given that they are close in proximity and that *R. heilongjiangensis* infection is prevalent in the Russian Far East (12). Further studies will be needed to clarify the relationship between *R. heilongjiangensis* and raccoons.

*R. felis* was first detected in the United States in 1990 (13) and has since spread throughout the world (14). Although the cat flea, *Ctenocephalides felis*, is currently the only known biological vector of *R. felis* (14), *R. felis* or *R. felis*-like DNA has been detected in several tick species, including those in Japan (15). More epidemiologic studies will be needed to confirm the identity of the *R. felis* vector.

Although *R. helvetica* was previously known to exist only in European countries (16), the pathogen has become widespread in Japan, from Hokkaido to the southern island of Kyushu (17). *Ixodes persulcatus* and *Ixodes ovatus* are possible vectors of *R. helvetica* in Japan (5). Feral raccoons in Hokkaido have been infected with *I. ovatus* and *I. persulcatus* (18); therefore, it is logical that *R. helvetica* DNA was detected in peripheral blood of raccoons in Hokkaido.

All rickettsial species detected in the present study, *R. heilongjiangensis* or *R. japonica*, *R. felis*, and *R. helvetica*, are pathogenic to humans (11,14,19). Because raccoons frequently approach areas where humans live, these *Rickettsia* spp. can infect humans via the tick vector introduced by raccoons. More epidemiologic studies are required to confirm the epidemiologic role of raccoons in *Rickettsia* infection.

In conclusion, this is the first report on the detection of SFG *Rickettsia* from peripheral blood of raccoons. Our results indicate that raccoons may be a reservoir for SFG *Rickettsia*.

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**Conflict of interest** None to declare.

**REFERENCES**


