Human *Rickettsia heilongjiangensis* Infection, Japan

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A case of *Rickettsia heilongjiangensis* infection in Japan was identified in a 35-year-old man who had rash, fever, and eschars. Serum contained *R. heilongjiangensis* antibodies, and eschars contained *R. heilongjiangensis* DNA. *R. heilongjiangensis* was also isolated from ticks in the suspected geographic area of infection.

Spotted fever group (SFG) rickettsiosis is the most prevalent arthropod-borne infectious disease in Japan (1). Before publication of a 1984 report about Japanese spotted fever (JSF) caused by *Rickettsia japonica*, scrub typhus caused by *Orientia tsutsugamushi* had been known as the sole rickettsiosis in Japan (1). Although many SFG *Rickettsia* species (*R. japonica*, *R. helvetica*, *R. tamurae*, *R. asiatica*, and other related *Rickettsia* spp.) were known, only *R. japonica* had been isolated or detected by PCR from Japanese SFG rickettsiosis patients (1–3). *R. japonica* was found in Dermacentor taiwanensis, Haemaphysalis cornigera, *H. flava*, *H. formosensis*, *H. hystricis*, *H. longicornis*, and Ixodes ovatus ticks, and *R. helvetica* in *H. japonica*, *I. columnae*, *I. monospinosus*, *I. ovatus*, *I. pavelovskyi*, *I. persulcatus*, and *I. turdus* ticks (3,4). Cases of SFG rickettsiosis caused by *R. heilongjiangensis*, showing mild rash associated with fever and an eschar, have been reported in the Russian Far East and the People’s Republic of China (5–8). In Russia and China, *R. heilongjiangensis* was isolated from *H. concinna* and *D. sylvarum* ticks (6,7). Highly related *Rickettsia* spp. were detected from *H. longicornis* ticks by PCR in South Korea (9). In this study, we confirmed a human case of *R. heilongjiangensis* infection in Japan. We also isolated *R. heilongjiangensis* from *H. concinna* ticks, a probable transmission vector, in the suspected geographic area of infection.

The Study

A 35-year-old man had chills and malaise on July 29, 2008 (day 0). On day 3, the patient became febrile (39.3°C). On day 5, a physician recognized the rash and prescribed oral minocycline (200 mg/d). On day 6, the patient was hospitalized because of constant fever and a whole body rash of unknown cause. At that time, laboratory data showed leukocyte count 7.2 × 10⁶ cells/L, thrombocyte count 275 × 10⁹ cells/L, aspartate aminotransferase 129 U/L, alanine aminotransferase 98 IU/L, and C-reactive protein 3.5 mg/dL. Biopsies were performed on eschars 1 and 2 (5–8 mm diameter) with erythema (∼20 mm diameter), above the left scapula and on the right lower back. During hospitalization, the patient received minocycline, 200 mg/day, intravenously. DNA was extracted from skin biopsy specimens by using a commercial kit according to the manufacturer’s instructions (Gentra Puregene; QIAGEN, Valencia, CA, USA). PCR was performed by using primers of 3 rickettsial genes: outer membrane protein A (*ompA*; primers Rr190.70p and Rr190.602n) (10), citrate synthase (*gltA*; primers Cs2d and CsEndr) (6), genus *Rickettsia*–specific outer membrane (17-kDa antigen gene) (11), and primers for *O. tsutsugamushi*, as reported previously (12).

Although many cases of *R. heilongjiangensis* infection show a single eschar as a result of a tick bite, *ompA*, *gltA*, and 17-kDa antigen genes were detected by PCR (but not with *O. tsutsugamushi*–specific primers) in both eschar specimens. Amplicons were sequenced and analyzed phylogenetically (Figure 1). The 491-bp fragment of *ompA* from eschar 1 (GenBank accession no. AB473995) demonstrated 99.8% and 97.1% nucleotide homology with *R. heilongjiangensis* strain HLJ-054 and *R. japonica* strain YM, respectively. The 1,250-bp fragment of *gltA* of eschar 1 (accession no. AB473991) demonstrated 99.9%, 99.8%, and 96.8% nucleotide homology with the *R. heilongjiangensis* strain C9P9, respectively. The 392-bp fragment of the 17-kDa antigen gene of eschar 1 (accession no. AB473987) demonstrated 100.0% and 99.2% nucleotide homology with the *R. heilongjiangensis* strain HLJ-054, *R. japonica* strain YM, and *R. helvetica* strain C9P9, respectively. The 392-bp fragment of the 17-kDa antigen gene of eschar 1 (accession no. AB473987) demonstrated 100.0% and 99.2% nucleotide homology with the *R. heilongjiangensis* strain HLJ-054 and *R. japonica* strain YM, respectively. Blood specimens were negative for rickettsial antigens by PCR, possibly because they were collected after minocycline treatment. Three serial blood samples were tested serologically by immunoperoxidase assays against rickettsial antigens: *R. japonica* strain YH;

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AB473996, AB473992, and AB473988, respectively; Sen-
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in Inner Mongolia, China, as an unknown SFG
our analysis was isolated from
CH8–1 (13). The R. heilongiangensis strain CH8–1 used in
our analysis was isolated from H. concinna ticks collected
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species (3). Strain CH8–1 was identified as R. heilongiangen-
sis by DNA analysis in this study (ompA, accession no.
AB473813; gltA, AB473812; and 17-kDa antigen genes,
AB473811). R. japonica and R. heilongiangensis antibody
titers were substantially elevated from day 6 to day 16.
Titers against R. heilongiangensis were 2–4 times higher
than titers against R. japonica on day 16 (Table).
An interview with the patient after the laboratory diag-
nosis of R. heilongiangensis infection revealed more infor-
mation about the context of the infection. He resided in an
urban area of Sendai, Miyagi Prefecture, Japan (Figure 2).
For 2 weeks before onset of symptoms, his outdoor activity
was limited to daily walking with a companion dog along a
river near his residence. The suspected area where he may
have become infected through a tick bite was investigated
in September 2008. We captured and examined 72
Hae-
maphysalis spp. ticks (52 H. longicornis, 15 H. concinna,
4 H. flava, and 1 H. megaspinosa) and 7 rodents (4 Rattus
norvegicus and 3 Microtus montebelli) for investigation
of SFG Rickettsia spp. Tick and rodent spleens were homog-
enized and subjected to isolation studies with L929 cells
in shell vial (3), and detection of Rickettsia DNA by PCR
was performed in parallel as previously described. Of the
72 tick samples, 3 H. concinna nymphs yielded Rickettsia
isolates and a DNA fragment of Rickettsia, which was de-
tected by PCR. Sequences of 3 isolates and amplicons were
identical to those from the patient’s specimens (Figure 1,
tick-derived isolates assigned Sendai-16, 29, 32; Sendai-
16: ompA, gltA, and 17-kDa antigen gene accession nos.
AB473996, AB473992, and AB473988, respectively; Sen-
dai-29: ompA, gltA, and 17-kDa antigen gene accession nos.
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Sendai-32: ompA, gltA, and 17-kDa antigen gene accession
nos. AB473998, AB473994, and AB473990, respectively).
PCR-detectable rickettsial agents were not isolated from
the rodents; however, 3 of the 4 R. norvegicus specimens
had high antibody titers to R. heilongiangensis.
To date, most cases of SFG rickettsiosis have been
reported as JSF in the western regions of Honshu Island,
Japan (1,2). R. japonica was isolated from ixodids in the
area where JSF is endemic, and R. helvetica from the en-
tire country of Japan (3). Moreover, only R. japonica has
been isolated from patients with SFG rickettsiosis (1,2).
A case-patient with JSF demonstrated serologic evidence of
SFG rickettsiosis caused by agents other than R. japonica;
however, those agents have not been defined (R. helvetica
in Fukui) (14). In 2007, another case of JSF was detected
serologically by using only R. japonica antigen in Aomori
Prefecture, the northernmost prefecture of Honshu Island
(15). However, R. japonica has not been detected in this
area (3). These results suggest that some cases of SFG rick-
ettsiosis in Japan may have been caused by SFG Rickettsia
species other than R. japonica.
The case reported in this article occurred in an urban
area of Sendai in the northern section of Honshu Island
(Figure 2). Scrub typhus caused by O. tsutsugamushi oc-

O. tsutsugamushi Karp, Kato, Gilliam, Kawasaki, Kuroki,
and Shimokoshi strains; and R. heilongiangensis strain
CH8–1 (13). The R. heilongiangensis strain CH8–1 used in
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The case reported in this article occurred in an urban
area of Sendai in the northern section of Honshu Island
(Figure 2). Scrub typhus caused by O. tsutsugamushi oc-
curs in this area with 2 seasonal peaks: from early spring to early summer, and from early fall to early winter (1). Serologic and microbiologic data ruled out scrub typhus in the present case. R. heilongjiangensis infection has been reported in the summer in the disease-endemic area of the Eurasian continent. Notably, the present case occurred in midsummer.

Conclusions

R. japonica has been the only known causative agent of SFG rickettsiosis in Japan, possibly because of limited availability of laboratory test systems. Further studies are needed to define the prevalence of SFG rickettsiosis caused by Rickettsia species other than R. japonica.

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