Case Report: *Borrelia valaisiana* Infection in a Japanese Man Associated with Traveling to Foreign Countries

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**Abstract.** A 78-year-old Japanese man who had clinical symptoms and a flu-like illness with fever, chills, diarrhea, and arthralgia had traveled to Cambodia and Khabarovsk, Russia, before the onset of symptoms and illness. He had been bitten by an *Ixodes persulcatus* tick in which the DNA of *Borrelia valaisiana* was detected. The patient’s symptoms improved rapidly after treatment with minocycline. Serologic examination detected antibodies to Lyme disease *Borrelia*. An flaB polymerase chain reaction with the patient’s plasma amplified a DNA fragment similar to that of *B. valaisiana*.

Lyme borreliosis is the most prevalent tick-borne zoonotic disease in Europe, North America, and the Far Eastern countries. Spirochetes belonging to the Lyme disease group are classified into 12 species. *Borrelia burgdorferi* is found in North America and Europe and is pathogenic to humans; *B. garinii* and *B. afzelii* are found in Europe and East Asian countries and are pathogenic to humans; *B. valaisiana*, *B. lusitaniae*, and *B. spielmanii* are found in *Ixodes ricinus* in Europe; *B. japonica*, *B. tanuki*, and *B. turdi* are found in *I. ovariatus*. *I. turdi*, respectively, in Japan; *B. andersonii* is found in *I. dentatus*; *B. bissetti* is found in *I. spinipalpis* in North America; and *B. sinica* is found in *I. ovariatus* in China.

The geographic distribution and enzootic transmission cycles of *Borrelia* species isolated in Japan have been well characterized. *Borrelia garinii* and *B. afzelii* are transmitted by *I. persulcatus* in Hokkaido and the northern half of Honshu, and *B. japonica* is transmitted by *I. ovariatus* in Hokkaido and most of Honshu. The Japanese strain Am501, which was isolated from the rare tick species *I. columnae*, has been identified as the *B. valaisiana* strain most commonly found in Europe. Takada and others reported *B. valaisiana* in *Mus calori* captured in the southernmost island of Japan, Okinawa, which is located in the subtropical zone. *B. valaisiana* was previously found in *I. nipponensis* in the Republic of Korea, in small wild mammals captured in Taiwan, and in small wild mammals and *L. granulatus* captured in China. Recently, *B. valaisiana*, which is widely distributed in the Far Eastern countries, was found in Japan. In the present report, we describe, to our knowledge, the first Japanese case of human infection with *B. valaisiana*.

On June 24, 2005, a 78-year-old Japanese man who had been treated for respiratory and heart disease was admitted to Tokyo University Hospital with febrility, fatigue, poor appetite, and cough. The patient had visited Cambodia and Khabarovsk, Russia, from June 10 to June 13, 2005, to hunt butterflies. He complained of a flu-like illness with fever, chills, diarrhea and arthralgia, but without swollen joints or erythema migrans. Hematologic examination showed a leukocyte count of 5,500/µL and a C-reactive protein (CRP) level of 4.48 mg/dL (normal < 0.5 mg/dL) on June 24. No abnormal results were found in other blood examinations. Although Cefotiam (2 g/day) was administered beginning on June 27, the patient’s condition did not improve, and the CRP level increased from 4.48 mg/dL to 9.83 mg/dL. A black tumor-like wart 10 mm in diameter was found at the right auricle in a subsequent medical examination and was recognized as a tick body by a dermatologist. Antibiotic treatment was changed from cefotiam to minocycline (200 mg/day) on June 30 because of the suspicion of *Borrelia* infection. Minocycline was continued until July 11. The patient’s symptoms improved rapidly and he left the hospital on July 12.

Blood (± 7 mL) and the tick body were collected on July 6, 2005 for serodiagnosis, bacterial cultivation, and polymerase chain reaction (PCR) examination. Serologic examination by immunoblotting was performed to detect antibodies to *B. burgdorferi* (strain B31), *B. garinii* (strain HP1 isolated from *I. persulcatus* in Hokkaido, Japan), and *B. afzelii* (strain P/Gau isolated from cerebrospinal fluid in Germany). Results for IgG against *B. garinii* were positive according to Centers for Disease Control and Prevention criteria. The IgM reacted strongly with 41-kD antigen (flagellin) of the *Borrelia* strains used for examination (Figure 1). To confirm seroreactivity, recomBlot *Borrelia* IgM (Mikrogen, Martinsried, Germany) was used. A recombinant 39-kD antigen reacted with the patient’s serum. The 39-kD antigen did not react under conventional immunoblotting methods with whole bacterial cells, which suggested that results were positive for IgM. Although it is recommended to test convalescent-phase serum samples, we were unable to carry out this test because the patient did not return to the hospital. Results of tests for antibodies to other arthropod-borne pathogens such as *Francisella tularensis*, *Brucella abortus*, *Rickettsia japonica* (the infectious agent of spotted fever rickettsiosis), *Rickettsia typhi* (the infectious agent of murine typhus rickettsiosis), and *Coelox burnetii* were negative.

Isolation of *Borrelia* from the whole blood was performed by inoculation into 10 mL of antibiotic-free Barbour-Stoenner-Kelly medium, which was prepared as described. The cultures were incubated at 33°C for 3 months; however, no spirochetes grew during the incubation period.
For PCR amplification, DNA was extracted from the patient’s blood and the tick body. Plasma and blood cells were obtained from 6 mL of EDTA-treated blood by centrifugation at 280 × g for 10 minutes. Plasma was centrifuged at 15,000 × g, and the pellet was used for isolation of DNA. Extraction of DNA from the sample was performed by using a High Pure PCR Template Purification Kit (Roche Diagnostics, Basel, Switzerland) following the manufacturer’s instructions. The DNA from the silica matrix was eluted with 100 μL of sterile water, and the eluted DNA solution was reduced into 20 μL by ethanol precipitation. Five microliters of the DNA solution was used for the PCR.

DNA extraction from the tick body was carried out by freezing the engorged tick body with liquid nitrogen and crushing. The DNA was extracted from the crushed tick body as described. To detect Borrelia DNA, we performed a nested flaB-PCR and an rrf-rrl intergenic spacer (RIS)-PCR, which identified Borrelia flaB-DNA and RIS-DNA fragments in the tick.

The patient’s plasma was positive for Borrelia by nested flaB-PCR, but negative by RIS-PCR. Amplified DNAs from tick and plasma were purified with a High Pure PCR Product Purification Kit (Roche Diagnostics), and sequencing was performed as described. The flaB sequence of the sample showed a high similarity value (98.6–97.4%) to that of B. valaisiana previously isolated in Far East countries and 96.0% DNA identity with B. valaisiana strain VS116 (GenBank accession no. D82854) (Table 1). It was experimentally confirmed that Lyme disease spirochetes were transiently present in the blood of the patient at a low density. In this study, the result of the nested flaB-PCR were positive but those of the RIS-PCR were negative, which may be explained by the ex-

**Table 1**

DNA identities in the flaB sequence of Borrelia detected in the present study compared with representative strains

<table>
<thead>
<tr>
<th>Strain (GenBank accession no.)</th>
<th>Country</th>
<th>Borrelia sp.</th>
<th>Base identities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKA1 (AB022132)</td>
<td>China</td>
<td>B. valaisiana</td>
<td>344/349 (98.6)</td>
</tr>
<tr>
<td>OMS8 (AB091705)</td>
<td>Japan</td>
<td>B. valaisiana</td>
<td>331/336 (98.5)</td>
</tr>
<tr>
<td>CMIN15 (AB022134)</td>
<td>China</td>
<td>B. valaisiana</td>
<td>343/349 (98.3)</td>
</tr>
<tr>
<td>QLM4P1 (DQ188928)</td>
<td>China</td>
<td>B. valaisiana</td>
<td>341/349 (97.7)</td>
</tr>
<tr>
<td>QLT1P2 (DQ188927)</td>
<td>China</td>
<td>B. valaisiana</td>
<td>341/349 (97.7)</td>
</tr>
<tr>
<td>CKA3a (AB022135)</td>
<td>China</td>
<td>B. valaisiana</td>
<td>340/349 (97.4)</td>
</tr>
<tr>
<td>VS116 (D82854)</td>
<td>Switzerland</td>
<td>B. valaisiana</td>
<td>335/349 (96.0)</td>
</tr>
<tr>
<td>I-77 (AF497995)</td>
<td>Czech</td>
<td>B. spielmanii</td>
<td>327/349 (93.7)</td>
</tr>
<tr>
<td>ACA1 (AB035613)</td>
<td>Sweden</td>
<td>B. afzelii</td>
<td>325/348 (93.4)</td>
</tr>
<tr>
<td>Ip90 (X75203)</td>
<td>Czech</td>
<td>B. garinii</td>
<td>325/349 (93.1)</td>
</tr>
<tr>
<td>B31 (AB035617)</td>
<td>USA</td>
<td>B. burgdorferi</td>
<td>323/348 (92.8)</td>
</tr>
<tr>
<td>NT112 (D82853)</td>
<td>Japan</td>
<td>B. japonica</td>
<td>326/349 (93.4)</td>
</tr>
</tbody>
</table>
perimental result that Lyme disease spirochetes were transiently present in the blood of the patient at a low density. This finding may also cause a false-negative result when the patient’s blood is examined by PCR.

To identify the tick species collected from the patient, we determined a partial sequence of the mitochondrial 16S rDNA gene (mt-rrs) of the tick and compared it with sequences in GenBank/European Molecular Biology Laboratory/DNA Data Bank of Japan because of its usefulness in tick species identification. The oligonucleotide primers 5’-CCGTCTGAACTCAGATCAAGTA-3’ and 5’-CTGCTCAATGATTTTTTAAATTGCTGTGG-3’ were used for amplification of the DNA fragment of mt-rrs, and sequencing of amplified products was carried out as described. The mt-rrs sequence of the tick was aligned with data deposited in GenBank/European Molecular Biology Laboratory/DNA Data Bank of Japan GenBank and the similarity value (%) was calculated. The DNA sequence of the tick showed a high similarity to that of I. persulcatus (99.2–100%), and other Ixodes ticks showed a similarity <93.5% (Table 2). On the basis of these results, the tick was identified as I. persulcatus.

We assume that the patient received the tick bite that caused Lyme disease by infection with B. valaisiana in Khabarovsky, Russia, because he had no history of visiting tick habitat areas in Japan before the onset of the disease, and because I. persulcatus has been documented in Khabarovsky but not in Cambodia. Borrelia valaisiana has been reported in the southeastern regions of Asia, such as southern China, Taiwan, the southern tip of the Korean Peninsula, and the Okinawa Islands in Japan. Our observations suggest that B. valaisiana is present in Russia, although, to the best of our knowledge, B. valaisiana has not been isolated in Khabarovsky. Thus, we do not rule out the possibility that the patient was infected in Cambodia or some other area and was later bitten by I. persulcatus in Khabarovsky.

To our knowledge, this is the first case report of human infection with B. valaisiana in Japan. It has been reported that the Borrelia species are widely distributed in countries in the Far East. On the basis of the present study, it is strongly recommended that people should take precautions against tick bites not only in regions where Lyme disease is endemic, but also in southeast Asia and other areas where ticks are infected with B. valaisiana.

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References


