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# Prevalence of Lyme Disease Spirochetes in *Ixodes persulcatus* and Wild Rodents in Far Eastern Russia

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**Borrelia spirochetes were isolated from the adult ixodid tick (*Ixodes persulcatus*) in three areas of far eastern Russia, namely, Khabarovsk, Vladivostok, and Yuzhno-Sakhalinsk. *Borrelia* infective rates of ticks in those areas were 24.5, 41.4, and 25.1%, respectively (total rate was 26.6%). Spirochetes were also isolated from the tissues of small mammals captured at Khabarovsk (infective rate was 20.8%). Samples were classified by rRNA gene restriction fragment length polymorphism (RFLP) analysis. The isolated spirochetes from ticks were classified mainly RFLP ribotype group IV (*B. garinii*), followed by groups II (*B. garinii*), III (*B. afzelii*), and V (*B. garinii*), showing that *B. garinii* is a dominant species among them. Both *B. garinii* and *B. afzelii* were also found in rodents, and multiple infections with those two species were observed in some rodents. *B. burgdorferi* sensu stricto (group I) was not isolated from either ticks or rodents.**

Lyme disease spirochetes are widely found in the northern hemisphere in North America, Europe, Russia, and East Asia. Within the Lyme disease *Borrelia* group *Borrelia burgdorferi* sensu lato, three human pathogenic species, *Borrelia burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* (2, 4), have been recognized. The distribution of those pathogens has been reported on a worldwide scale; *B. burgdorferi* sensu stricto is found in North America and Europe, and *B. garinii* and *B. afzelii* are found in Europe, Russia, and East Asia (1, 8, 9, 11, 12, 15). However, little is known about which *Borrelia* species exist in far eastern Russia. The main vector of Lyme disease in this area, *Ixodes persulcatus*, can also mediate tick-borne encephalitis virus. The etiology of tick-borne encephalitis was established in 1937; however, that of Lyme disease was reported in 1985 (10). The Lyme disease spirochete has been detected in the Khabarovsk territory with a 20% infection rate in 1989 (11), but classification of the borrelia within the ticks has not been described sufficiently. Wild rodents such as *Apodemus* and *Clethrionomys* spp. are considered to be reservoirs of the disease in the area, but the spirochetes from these rodents have not been isolated. Furthermore, human cases of this spirochetal infection have not been clear in this region. Official records of Lyme disease in Russia have been kept only since 1992 (10); the infection rate would be speculative. In this study, to evaluate the infective rate of ticks and small mammals and to characterize the Lyme disease spirochetes in the area, we isolated a large number of spirochetes from collected ticks and rodents and classified the samples by rRNA gene restriction fragment length polymorphism (RFLP) analysis.

Adult *I. persulcatus* ticks were collected by flagging vegetation at three different woodland areas in far eastern, Russia namely, Khabarovsk, Vladivostok, and Yuzhno-Sakhalinsk, from the end of May to the beginning of June 1995. At the same time, small mammals were also captured by snap traps in Khabarovsk. The midgut of each tick and small tissues of both earlobes, heart muscles, and urinary bladders from individual feral animals were inoculated in BSK II medium (3) and then cultured at 31°C for 4 weeks as previously described (12). Spirochetal DNAs were prepared as described previously (7) and digested with *Eco*RV or *Hin*cII (Takara Biochemicals, Kyoto, Japan). Resulting DNA fragments were electrophoresed in 0.7% agarose gel with TAE buffer (40 mM Tris, 20 mM sodium acetate, 30 mM acetic acid, 2 mM EDTA [pH 7.8]) and transferred to a nylon membrane (Hybond-N+; Amersham International, Tokyo, Japan) by the method of Southern (16). The blotted DNAs were bound to the membrane by UV cross-linking. An enhanced chemiluminescence kit (ECL random prime labeling and detection systems; Amersham) was used for hybridization. The 23S rRNA gene fragments of *B. burgdorferi* B31, designated NP and Sty (7), were labeled with fluorescein-11-dUTP and used as probes. The bound membranes were prehybridized and then incubated with denatured labelled probes overnight at 60°C for hybridization. The blots were washed in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) after a wash in 0.5× SSC and then were blocked with blocking agent solution and incubated with peroxidase-labelled anti-fluorescein antibody diluted 1/1,000 for 1 h. After rinsing of the membranes with 0.1% Tween 20 in antibody wash buffer, substrate solution was added, and then the blots were exposed to X-ray films (Hyperfilm-ECL).

Table 1 summarizes the results of isolation of spirochetes from ticks. Coleman et al. (5) isolated 11 spirochetes from *Ixodes ricinus* and *I. persulcatus* in the area of the former Soviet Union. They reported that isolated spirochetes from *I. persulcatus* in Khabarovsk were categorized as *B. garinii* ($n = 2$), but the total number of samples was not sufficient to estimate the prevalence of borrelia spirochetes in the area. In this study, we examined 846 adult ticks and found a total of 225 spirochetal samples. The spirochete infective rate in ticks was the highest

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TABLE 1. Isolation of spirochetes from ticks in far eastern Russia

| Location | No. of ticks infected/no. examined (%) |
|---|---|
| Khabarovsk | 138/564 (24.5) |
| Vladivostok | 41/99 (41.4) |
| Yuzhno-Sakhalinsk | 46/183 (25.1) |
| Total | 225/846 (26.6) |

at Vladivostok (41.4%) and was 26.6% on average in the three regions. In Khabarovsk, we also captured four species of rodents and one insectivore, consisting of 4 striped field mice (*Apodemus agrarius*), 10 Korean field mice (*Apodemus peninsulae*), 25 gray red-backed voles (*Clethrionomys rufocanus*), 8 Siberian chipmunks (*Tamias sibiricus*), and one shrew (*Sorex* sp.). Spirochetes were isolated from two mice (*A. peninsulae*) and eight voles (*C. rufocanus*). The total spirochete-positive rate of small mammals was 20.8% (10 of 48 animals examined).

The grouping scheme of borrelia spirochetes with rRNA gene RFLP ribotyping was modified from that described by Fukunaga et al. (7). We examined 54, 22, and 19 of the samples from ticks collected in Khabarovsk, Vladivostok, and Yuzhno-Sakhalinsk, respectively (Table 2). All of the spirochetes examined had two repeated copies of the 23S and 5S rRNA genes, which is a unique organization for the Lyme disease pathogen. The spirochetes from ticks were mainly classified as group IV (47.4%), followed by group II (11.6%), group III (11.6%), and group V (3.2%). Other samples with mixed patterns or RFLP patterns that did not fit the scheme described above were categorized as "Others" (26.3%). Some samples showed mixed RFLP patterns of two ribotypes. The compositions of mixed RFLP patterns observed were IIc + IVa, IVa + IVb, IVa + IVc, IVa + V, and IVc + V, respectively. There were no sets of ribotype groups such as IIIc + IVa, IIIc + IVb, or IIIc + IVc. RFLP patterns of ribotypes that did not fit the scheme described above were different from each other, so they could not be accumulated in several groups. Ribotype groups I, II, and III corresponded to *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*, respectively (7), and groups IV, V, and VI are now considered to be the species *B. garinii* (6). Consequently, our findings indicate that *B. garinii* is a dominant species among infective ticks (62.1% in total) in the three regions studied. Spirochetes isolated from rodents were also classified as mainly *B. garinii*, followed by *B. afzelii*. In addition to spirochetes from ticks, we found mixed RFLP patterns of two ribotypes in rodents but no pairs such as ribotypes II and III or ribotypes III and IV, which means that there is no coexistence of both *B. garinii* and *B. afzelii* in any sample. On the other hand, in some parts of the organs, some rodents had different *Borrelia* species (Table 3), suggesting the occurrence of multiple infections. Similarly, Nakao and Miyamoto (13) reported such multiple infections in *Apodemus speciosus* mice in Hokkaido, northern Japan. Ribotypes of neither *B. burgdorferi* sensu stricto nor *B. japonica* were found in spirochetes isolated from ticks or rodents. These ribotypes in far eastern Russia may resemble those of spirochetes isolated in Hokkaido (14) in that *B. garinii* is a dominant species among the samples there. More detailed studies may provide epidemiological findings to help us understand the course of development of Lyme disease spirochetes, the vectors, and the reservoirs in the Far East areas.

TABLE 2. Frequency of ribotype groups among spirochetes isolated from ticks in far eastern Russia

| Species and ribotype group | No. (%) of spirochetes at: | | |
|---|---|---|---|
| | Khabarovsk | Vladivostok | Yuzhno-Sakhalinsk |
| *B. burgdorferi* sensu stricto I | 0 | 0 | 0 |
| *B. garinii* | | | |
| IIa | 3 (5.6) | 3 (13.6) | 0 |
| IIb | 0 | 0 | 0 |
| IIc | 5 (9.3) | 0 | 0 |
| IVa[a] | 12 (22.2) | 9 (40.9) | 9 (47.4) |
| IVb[a] | 8 (14.8) | 1 (4.5) | 2 (10.5) |
| IVc[a] | 3 (5.6) | 1 (4.5) | 0 |
| V[a] | 1 (1.9) | 1 (4.5) | 1 (5.3) |
| VI[a] | 0 | 0 | 0 |
| *B. afzelii* | | | |
| IIIa | 0 | 1 (4.5) | 0 |
| IIIb | 0 | 0 | 0 |
| IIIc | 6 (11.1) | 3 (13.6) | 1 (5.3) |
| Others[b] | 16 (29.6) | 3 (13.6) | 6 (31.6) |
| Total no. of spirochetes examined | 54 | 22 | 19 |

[a] These groups were previously considered unknown species.
[b] The category "Others" includes spirochetes with mixed RFLP patterns of ribotypes or uncategorized ribotypes that do not fit the scheme.

TABLE 3. RFLP ribotyping of spirochetes from rodents captured at Khabarovsk

| Rodent | Ribotype from cultured tissue[a] | | | |
|---|---|---|---|---|
| | Left ear lobe | Right ear lobe | Heart muscle | Urinary bladder |
| *A. peninsulae*[b] | IVb | Mixed | IVa | IVa |
| *A. peninsulae* | IVa | — | — | IVc |
| *C. rufocanus*[c] | Mixed | Mixed | IIIc | IVb |
| *C. rufocanus* | IVb | Mixed | IIIc | Mixed |
| *C. rufocanus* | IVb | IVb | Mixed | — |
| *C. rufocanus* | IVc | Mixed | Mixed | IIIc |
| *C. rufocanus* | — | — | IVa | — |
| *C. rufocanus* | Mixed | — | IVb | — |
| *C. rufocanus* | Mixed | Mixed | Mixed | Mixed |
| *C. rufocanus* | IVa | IIIc | IVa | IVa |

[a] Ribotype IIIc corresponds to *B. afzelii*; ribotypes IVa, IVb, and IVc correspond to *B. garinii*. Mixed, mixture of plural RFLP patterns; —, no spirochetes detected.
[b] Korean field mouse.
[c] Gray red-backed vole.

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