JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2176–2181 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2176–2181.2002

Genetic Identification of Rickettsiae Isolated from Ticks in Japan

Pierre-Edouard Fournier,[1] Hiromi Fujita,[2] Nobuhiro Takada,[3] and Didier Raoult[1]*

Unité des Rickettsies, Faculté de Médecine, CNRS UMR 6020 27, Université de la Méditerranée, 13385 Marseille, Cedex 05, France,[1] and Ohara Research Laboratory, Ohara General Hospital, Fukushima 960-0195,[2] and Department of Immunology and Medical Zoology, Fukui Medical University, Matsuoka, Fukui 910-1193,[3] Japan

Received 7 January 2002/Returned for modification 2 March 2002/Accepted 24 March 2002

Following the description in Japan of Japanese spotted fever, caused by *Rickettsia japonica*, a search for the vector of this disease led to the isolation of several rickettsiae from various tick species. Sixty-three rickettsial isolates were obtained from six different tick species, and six type strains were described by PCR and monoclonal antibody testing. We identified these six strains by amplification and sequencing of the genes encoding 16S rRNA and citrate synthase. We confirmed that the isolates from *Dermacentor taiwanensis* and *Haemaphysalis flava* ticks were *R. japonica* isolates. In *Ixodes ovatus*, *Ixodes persulcatus*, and *Ixodes monospinosus*, we identified a *Rickettsia* identical or closely related to *Rickettsia helvetica*, a species that is pathogenic for humans and that to date has only been found in Europe. Finally, we identified a new genotype of unknown pathogenicity, genotype AT, that was isolated from *Amblyomma testudinarium* ticks and that is closely related to a Slovakian genotype obtained from *Ixodes ricinus* ticks.

Rickettsioses are emerging infectious diseases caused by rickettsiae, which are obligate intracellular gram-negative bacteria associated with arthropod parasites. These arthropod vectors (ticks, fleas, mites, and insects) can transmit rickettsiae to mammals and humans. The rickettsioses share characteristic clinical features, including fever, headache, rash, and sometimes eschar (tache noire) formation at the site of the tick bite. The number of representatives of the genus *Rickettsia* and the number of newly described rickettsioses have increased in recent decades as a result of the development of an improved cell culture isolation technique and the extensive use of bacterial detection and identification based on molecular biology techniques (14). Comparison of the sequences of PCR-amplified fragments of the genes encoding 16S rRNA (ribosomal DNA [rDNA]) (16), citrate synthase (*gltA*) (17), or the rOmpA outer membrane protein (*ompA*) has become a reliable method for the identification of *Rickettsia* spp. (15).

The discovery of Japanese spotted fever by Mahara (9) in 1984 began the study of spotted fever group (SFG) rickettsioses in Japan. The causative agent was identified and named *Rickettsia japonica* (22). The bacterium was apparently detected in three genera and six species of ticks (9). This stimulated the search for rickettsiae in ticks (19); and since 1993, 63 strains have been isolated from six tick species: *Amblyomma testudinarium* (25 isolates) (5, 25), *Dermacentor taiwanensis* (1 isolate) (20, 24), *Haemaphysalis flava* (1 isolate) (6), *Ixodes monospinosus* (1 isolate) (4), *Ixodes ovatus* (33 isolates), and *Ixodes persulcatus* (2 isolates) (4). These isolates were tested by using two monoclonal antibodies (MAbs) and the immunoperoxidase reaction. One of the two MAbs, which had a broad spectrum of reactivity, reacted with all SFG rickettsiae, and the other MAb was specific for *R. japonica* (12). These isolates were also tested by PCR with primer sets derived from *gltA*, *ompA*, and *ompB*. The *gltA* gene was amplified from all strains, but the other two genes were amplified from only certain rickettsiae (Fig. 1). On the basis of the results of these tests, two new species were identified, the AT-type *Rickettsia* sp. (25 isolates from *A. testudinarium*; isolates AT-1 to AT-25) and the IO-type *Rickettsia* sp., including 33 isolates from *I. ovatus* (isolates IO-1 to IO-33) as well as the single isolate from *I. monospinosus* (isolate IM-1) and 2 isolates from *I. persulcatus* (isolates IP-1 and IP-2). Isolate DT-1, isolated from *D. taiwanensis*, and isolate FLA-1, isolated from *H. flava*, were considered *R. japonica* strains on the basis of MAb testing.

The purpose of the present work was to determine by PCR amplification and sequencing of the 16S rDNA and *gltA* genes the current positions of six typical Japanese isolates (isolates AT-1, IO-1, DT-1, IM-1, FLA-1, and IP-1) and estimate their potential roles as human pathogens.

MATERIALS AND METHODS

Strains AT-1, IO-1, DT-1, IM-1, FLA-1, and IP-1 were initially cultivated in L929 cells (4) (Table 1). They were subsequently propagated in Vero cells as described previously (8). Their DNA was extracted with the QIAamp tissue kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. PCR amplification and sequencing reactions were performed with oligonucleotide primers derived from the 16S rDNA and *gltA* genes (Table 2). All primers were purchased form Eurobio (Les Ulis, France), and amplifications were carried out in a Peltier model PTC-200 DNA thermocycler (MJ Research, Inc., San Francisco, Calif.) under previously described conditions (17). Each amplicon obtained was purified for sequencing with a QIAquick Spin PCR Purification kit (Qiagen, Courtaboeuf, France) by the protocol described by the manufacturer. Sequencing reactions were carried out with a D-rhodamine terminator cycle DNA sequencing kit (Applied Biosystems, Foster City, Calif.) as described by the manufacturer. Sequencing reaction products were resolved by electrophoresis with an ABI Prism 377 sequencer (Applied Bioystems). The results obtained were processed into sequence data by using sequence Navigator and AutoAssembler software. Each base position was established at least three times in both the forward and the reverse directions.

The sequences of the 16S rDNA and *gltA* genes were aligned by use of the multisequence alignment program CLUSTAL within the BISANCE environment. Phylogenetic relationships between the six strains and other SFG rickettsiae were inferred by using version 3.4 of the PHYLIP software package (2). The distance matrices generated by DNADIST were determined under the assump-

* Corresponding author. Mailing address: Unité des Rickettsies, Faculté de Médecine, CNRS UMR 6020, Université de la Méditerranée, 27, Blvd. Jean Moulin, 13385 Marseille, Cedex 05, France. Phone: 33 4 91 32 43 75. Fax: 33 4 91 38 77 72. E-mail: Didier.Raoult@medecine.univ-mrs.fr.

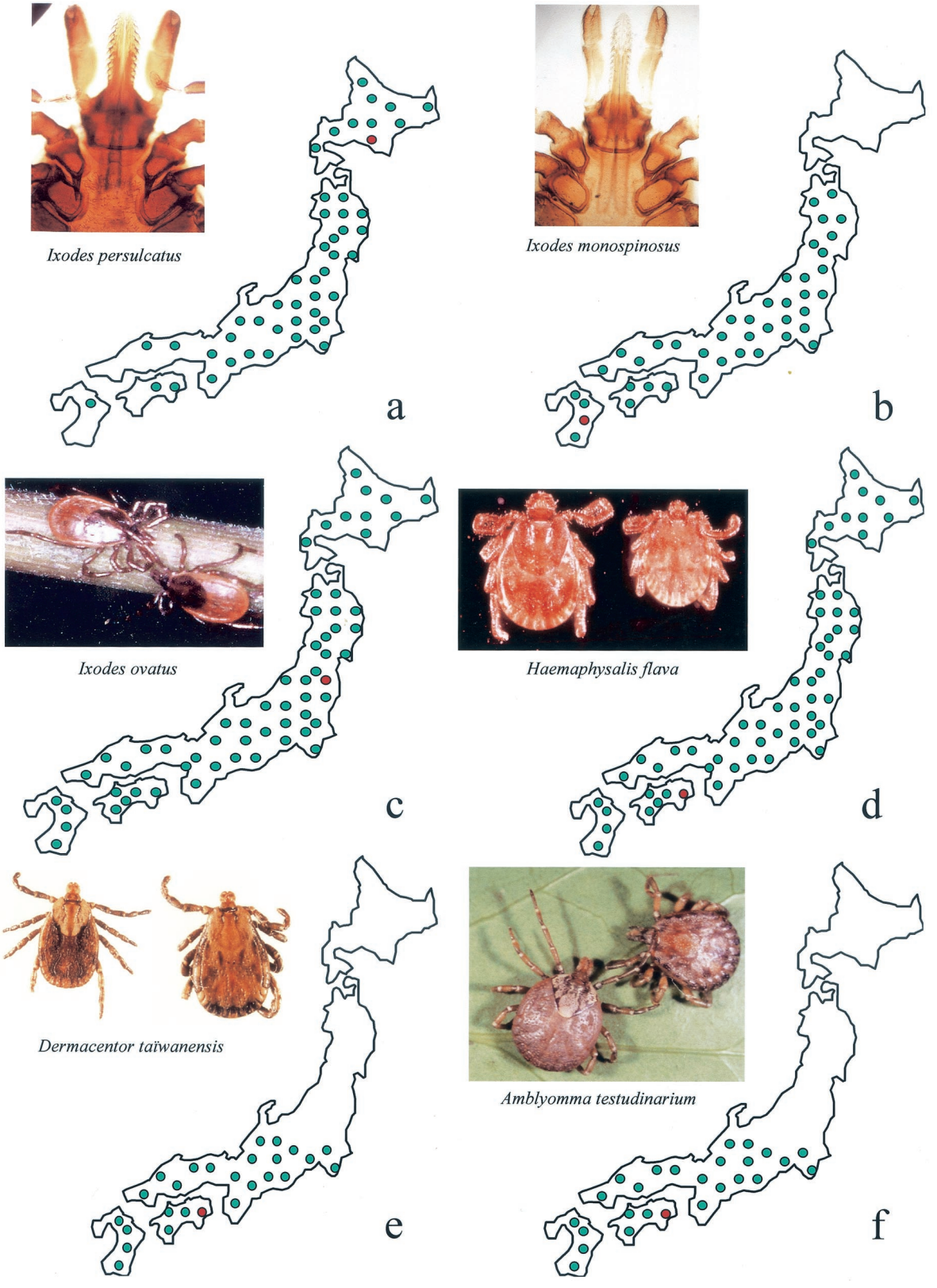

FIG. 1. Geographical distributions in Japan of the ticks from which the rickettsial isolates studied were isolated. (a) *I. persulcatus*; (b) *I. monospinosus*; (c) *I. ovatus*; (d) *H. flava*; (e) *D. taiwanensis*; (f) *A. testudinarium*. Green dots indicate the distributions of the ticks, and the red dots indicate the area where the ticks harboring the rickettsiae were collected.

TABLE 1. Descriptions of the six rickettsial strains isolated from Japanese ticks[a]

Strain	Tick species	Location	Yr of isolation	Reference	Polyclonal IQ-1 serotype	Reactivity with MAb specific for:		PCR amplification	
						SFG rickettsiae (MAb S3)	*R. japonica* (MAb C3)	Rr-90-70p-602n (rOmpA)	BG1-21-BG2-20 (rOmpA)
AT-1	*A. testudinarium*	Western Japan	1993	6	−	+	−	+	−
DT-1	*D. taiwanensis*	Western Japan	1993	20	−	+	+	+	+
IM-1	*I. monospinosus*	Southern Japan	1993	6	+	+	−	−	−
FLA-1	*H. flava*	Western Japan	1998	6	−	+	+	?	?
IO-1	*I. ovatus*	Central Japan	1993	4	+	+	−	−	−
IP-1	*I. persulcatus*	Northern Japan	1966	4	+	+	−	−	−

[a] The rickettsial strains were cultivated in L929 cells.

tions of Kimura (6a) and were used to infer dendrograms by the neighbor-joining method. Dendrograms were also constructed by data processing with the maximum-likelihood and parsimony programs in the PHYLIP software package. A bootstrap analysis based on 100 randomly generated trees by using SEQBOOT and CONSENSE in the PHYLIP software package was performed to estimate the node reliabilities of the trees obtained by three phylogenetic methods (1).

Nucleotide sequence accession numbers. The GenBank nucleotide sequence accession numbers for the 16S rDNA sequences of isolates DT-1, FLA-1, IP-1, IM-1, IO-1, and AT-1 are AF394902, AF394903, AF394904, AF394905, AF394906, and AY049981, respectively; the GenBank nucleotide sequence accession numbers for the *gltA* sequences of DT-1, FLA-1, IP-1, IM-1, IO-1, and AT-1 are AF394897, AF394898, AF394899, AF394900, AF394901, and AF394896, respectively.

RESULTS

For all six strains studied, the 16S rDNA sequence was 1,440 nucleotides long, whereas the *gltA* sequence was 1,234 nucleotides long in all strains except strain AT-1, which exhibited a 1,235-nucleotide *gltA* sequence due to the insertion of an adenine at position 691. The sequences of the 16S rDNA and *gltA* genes of IP-1 and IM-1 exhibited 100% homology with those of *R. helvetica*, whereas the sequences of the 16S rDNA and *gltA* genes of IO-1 differed from those of *R. helvetica* by 14 and 12 base substitutions, respectively (Table 3). The sequences of the 16S rDNA and *gltA* genes of FLA-1 were 100% homologous to those of *R. japonica*, but the sequences of the 16S rDNA and *gltA* genes of DT-1 differed from those of *R. japonica* by three base substitutions for both genes (Table 3). The sequence of the 16S rDNA gene of AT-1 differed from those of isolates Slovakia 3 and Slovakia 4 (obtained from *Ixodes ricinus* ticks) by 11 and 10 base substitutions, respectively, and the sequence of the *gltA* gene of AT-1 differed from those of Slovakia 3 and Slovakia 4 by 11 and 13 base substitutions, respectively (Table 3).

On the basis of analysis of the 16S rDNA and *gltA* sequences, IP-1, IM-1, and IO-1 clustered with *Rickettsia helvetica*; and this cluster was supported by a 100% bootstrap value. The cluster made up of FLA-1, DT-1, and *R. japonica* was also supported statistically. AT-1 was reliably related to uncultivated isolates Slovakia 3 and Slovakia 4 from *I. ricinus* ticks (18). The phylogenetic classifications of the other rickettsial species were similar to those obtained previously (17) (Fig. 2).

DISCUSSION

Only one spotted fever rickettsiosis, Japanese spotted fever, caused by *R. japonica*, has been described in Japan (9). However, since 1993, 63 rickettsial isolates, which have been classified into six species on the basis of identical gene sequences or reactivities to MAbs, have been cultivated from six tick species (4, 6, 20, 24, 25). In the work described here, we analyzed one isolate from each of these six rickettsial species. We identified isolates IP-1 and IM-1 as *R. helvetica* strains, isolate IO-1 as being closely related to but different from *R. helvetica*, and isolates FLA-1 and DT-1 as *R. japonica* strains. In addition, we found that isolate AT-1 is a new genotype.

R. helvetica was first isolated in Switzerland from *I. ricinus*, the vector of Lyme disease in Europe. Later, it was found in France, Portugal, and Sweden. In this work, it was isolated from *I. ovatus*, the vector of *Borrelia japonica* in Japan. Thirty-three strains were isolated from *I. ovatus* ticks, two strains were isolated from *I. persulcatus* ticks, and one strain was isolated from an *I. monospinosus* tick; all of these strains belonged to the same serotype. At present, *R. helvetica* is present only in *I. ricinus* ticks in Europe, and our data show for the first time that the distribution of this bacterium is not limited to Europe but extends to Asia. As *I. persulcatus*, the vector of Lyme disease in Japan (21), belongs to the *I. ricinus* complex, one could expect the area of distribution of *R. helvetica* in Eurasia to be close to that of *Borrelia burgdorferi* sensu lato. In Europe, forest workers in areas where *I. ricinus* is prevalent have high seroprevalences of antibodies to *R. helvetica* (3). In Japan, *I. persulcatus* tick bites have been reported (7). Three patients reported similar fingernail-sized erythemas at the site of the tick bite. However, the role of *R. helvetica* in this manifestation was not

TABLE 2. Oligonucleotide primers used in the study

Primer name	Nucleotide sequence (5′-3′)	*gltA* positions relative to the open reading frame[a]
CS1d[b]	ATG ACT AAT GGC AAT AAT AA	1–20
CS890r[b,c]	GCT TTA GCT ACA TAT TTA GG	890–871
Rp877p[b,c]	GGG GAC CTG CTC ACG GCG G	797–815
Rp1258n[b,c]	ATT GCA AAA AGT ACA GTG AAC A	1178–1157
CS1273r[b,c]	CAT AAC CAG TGT AAA GCT G	1273–1255
CS113d[c]	GTA GGG TAT CTG CGG AAG C	113–131
CS409d[c]	CCT ATG GCT ATT ATG CTT GC	409–428
CS535d[c]	GCA ATG TCT TAT AAA TAT TC	535–554
CS1048d[c]	CTT GAA GCT CTC CGC TCT TAA	1048–1067
CS244r[c]	CTT TAA TAT CAT ATC CTC GAT	144–224
CS428r[c]	GCA AGC ATA ATA GCC ATA GG	428–409

[a] Numbering refers to the *gltA* sequence of *R. prowazekii* (23).
[b] Oligonucleotide primer used for PCR amplification.
[c] Oligonucleotide primer used for sequencing.

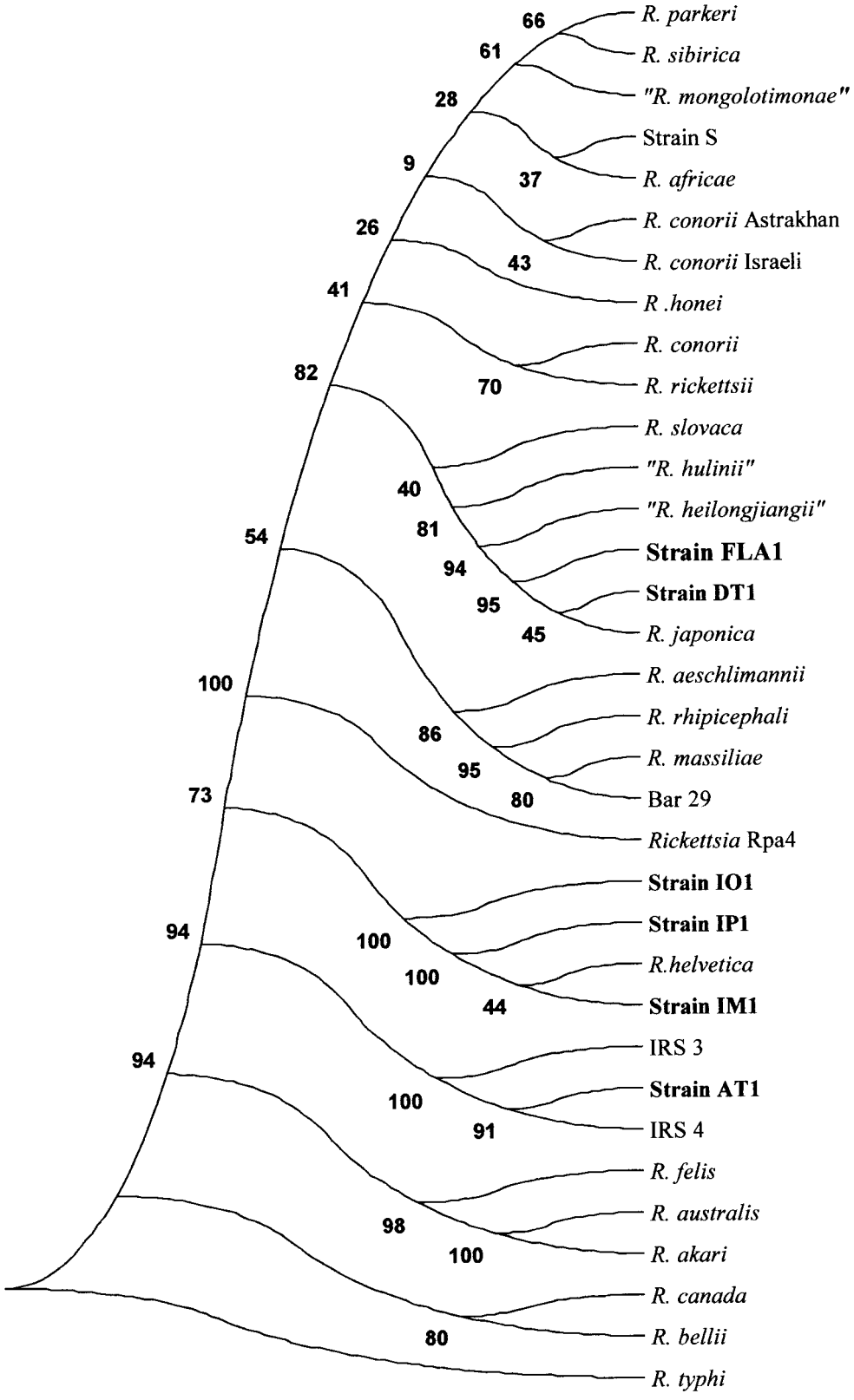

FIG. 2. Citrate synthase gene-based phylogenetic tree showing the positions among the rickettsiae of the six Japanese strains described in this study. The tree was constructed by the parsimony method. Bootstrap values are indicated at the nodes.

TABLE 3. Nucleotide differences between the six Japanese rickettsial isolates and their closest relative species[a]

Rickettsial isolate	Nucleotide differences by comparison with:	16S rDNA	*gltA*
IP-1	*R. helvetica*	No difference	No difference
IM-1	*R. helvetica*	No difference	No difference
IO-1	*R. helvetica*	68 (T→C), 73 (G→A), 124 (T→C), 243 (G→A), 302 (G→T), 388 (G→A), 441 (C→T), 474 (G→T), 942 (C→T), 1059 (A→G), 1085 (A→G), 1132 (C→T), 1356 (T→A), 1361 (G→A)	8 (G→A), 86 (A→G), 133 (A→G), 181 (T→G), 202 (A→G), 415 (T→G), 609 (C→T), 730 (T→C), 793 (T→C), 832 (T→C), 889 (G→A), 1144 (G→T)
FLA-1	*R. japonica*	No difference	No difference
DT-1	*R. japonica*	749 (G→C), 766 (C→T), 1390 (C→G)	771 (C→G), 784 (T→C), 1134 (A→T)
AT-1	Slovakia 4 from *I. ricinus*	37 (A→G), 44 (C→T), 45 (T→C), 91 (A→G), 162 (A→G), 163 (A→G), 688 (A→G), 693 (T→A), 815 (A→G), 972 (G→A)	16 (C→A), 19 (G→A), 160 (A→G), 235 (A→G), 288 (G→A), 528 (A→G), 605 (A→G), 619 (G→A), 691[b] (none→A), 908 (T→C), 974 (A→G), 988 (A→G), 1145 (G→T)
AT-1	Slovakia 3 from *I. ricinus*	37 (A→G), 44 (C→T), 45 (T→C), 91 (A→G), 162 (A→G), 163 (A→G), 688 (A→G), 693(T→A), 815 (A→G), 972 (G→A), 1219 (A→none)	16 (C→A), 160 (A→G), 235 (A→G), 288 (G→A), 528 (A→G), 605 (A→G), 619 (G→A), 691[b] (none→A), 974 (A→G), 988 (A→G), 1145 (G→T)

[a] Nucleotide positions are relative to those of the 16S rDNA and *gltA* sequences of *R. helvetica* (GenBank accession numbers L36212 and U59723, respectively), *R. japonica* (GenBank accession numbers L36213 and U59724, respectively), Slovakia 4 from *I. ritinus* (GenBank accession numbers AF141908 and AF141906, respectively), and Slovakia 3 from *I. ricinus* (GenBank accession numbers AF141907 and AF140706, respectively). Substitutions are indicated as nucleotide in reference species → nucleotide in Japanese isolate.

[b] The nucleotide difference at position 691 is an insertion.

been demonstrated. Recently, another Japanese patient developed similar symptoms following an *I. persulcatus* bite and seroconverted to positivity for antibodies to *R. helvetica* (H. Inokuma and D. Raoult, unpublished data). At present, the pathogenic role of *R. helvetica* has been demonstrated in two articles: in France, a man who had an isolated episode of fever and who had been exposed to *I. ricinus* seroconverted to positivity for antibodies to *R. helvetica* (3), and in Sweden, two patients with sudden death suffered perimyocarditis caused by *R. helvetica*, as determined by electron microscopy, PCR, and serology (11).

R. japonica is the agent of Japanese spotted fever. In the present work, we confirm that *D. taiwanensis* and *H. flava* harbor isolates of *R. japonica*. By using MAbs and PCR, this species was also identified in *I. ovatus* ticks, which is discrepant with our results. We believe that *R. japonica* may be present throughout the areas of distribution of *D. taiwanensis* (eastern China and Taiwan) and *H. flava* (Korea, China, and Taiwan). Very closely related organisms were reported in China (26) and were isolated from *Dermacentor silvarum* ticks (isolate 054, or "*Rickettsia heilongjiangii*") and *Haemaphysalis concinna* ticks (isolate HL-93, or "*Rickettsia hulinii*"). The spectrum of infected ticks is large, which is unusual for tick-transmitted rickettisae except *R. rickettsii* (14). It is also surprising that the geographical distribution of Japanese spotted fever is much more restricted than that of its vector tick. Usually, for SFG rickettsiae, ticks are both the reservoir and the vector of the bacteria (14) and the geographical distribution of the disease is superposed on that of the tick. The notable exception to this is *R. conorii*, which is absent in the Americas, despite the presence of *Rhipicephalus sanguineus*, its reservoir tick (13). As is the case for *R. japonica*, the lack of evidence of Japanese spotted fever outside southern Japan may be caused either by a lack of infection or by the absence of recording of the disease.

We identified a new genotype in a rickettsial organism isolated from *A. testudinarium* (isolate AT-1). This genotype is related to that for two rickettsiae previously identified in *I. ricinus* ticks collected in Slovakia only by PCR amplification and sequencing (18). *A. testudinarium* ticks frequently bite humans in Japan (9, 10) and could be a potent vector of rickettsial diseases. Recently, a case of pseudo-Lyme disease characterized by a skin lesion and local enlarged lymph nodes was observed in Belgium in a patient returning from Nepal. An *A. testudinarium* tick was found on the skin lesion (P. Van Gompel, unpublished data). However, the causative role of AT-1 in this patient remains to be demonstrated.

In conclusion, we confirmed that the rickettsiae isolated from *D. taiwanensis* and *H. flava* ticks were *R. japonica* strains, and thus, these tick species are potential vectors of Japanese spotted fever. We identified *R. helvetica* outside Europe for the first time and identified a new genotype of rickettsia in *A. testudinarium* ticks. As the vector ticks easily bite humans, these rickettsiae represent a potential threat in Japan. Because the ticks analyzed are also prevalent in other areas of Central and Eastern Asia, the distributions of these rickettsiae may well be much larger.

ACKNOWLEDGMENT

We thank Marie-José Casagrande for helpful contributions.

REFERENCES

1. **Brown, J. K. M.** 1994. Bootstrap hypothesis tests for evolutionary trees and other dendograms. Proc. Natl. Acad. Sci. USA **91:**12293–12297.
2. **Felsenstein, J.** 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics **5:**164–166.
3. **Fournier, P. E., F. Gunnenberger, B. Jaulhac, G. Gastinger, and D. Raoult.** 2000. Evidence of *Rickettsia helvetica* infection in humans, eastern France. Emerg. Infect. Dis. **6:**389–392.
4. **Fujita, H., N. Takada, E. Isogai, Y. Watanabe, and T. Ito.** 2000. Isolation of spotted fever group rickettsiae from *Ixodes persulcatus* ticks in the central part of Hokkaido, Japan. Med. Entomol. Zool. **51:**55–58.
5. **Fujita, H., N. Takada, and Y. Tsuboi.** 1996. Survey of ixodid ticks (Acarina: Ixodidae) and tick-borne spotted fever group rickettsiae in Tokunoshima Island, Japan. Med. Entomol. Zool. **47:**15–21.
6. **Fujita, H., Y. Watanabe, M. Ishikura, and N. Takada.** 1999. List of all isolates of spotted fever group rickettsiae from ticks in Japan 1993–1998. Annu. Rep. Ohara Hosp. **42:**45–50.

6a. **Kimura, M.** 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:**111–120.

7. **Kumazawa, H., T. Saruta, and N. Takada.** 1998. Three human cases of tick bite caused by *Ixodes persulcatus* at high altitude in Shikoku Island, Japan. Acarol. Soc. Jpn. **7:**51–54.
8. **La Scola, B., and D. Raoult.** 1997. Laboratory diagnosis of rickettsioses: current approaches to the diagnosis of old and new rickettsial diseases. J. Clin. Microbiol. **35:**2715–2727.
9. **Mahara, F.** 1997. Japanese spotted fever: report of 31 cases and review of the literature. Emerg. Infect. Dis. **3:**105–111.
10. **Nakamura-Uchiyama, F., Y. Komuro, A. Yoshii, and Y. Nawa.** 2000. Amblyomma testudinarium tick bite: one case of engorged adult and a case of extraordinary number of larval tick infestation. J. Dermatol. **27:**774–777.
11. **Nilsson, K., O. Lindquist, and C. Pahlson.** 1999. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet **354:** 1169–1173.
12. **Oikawa, Y., N. Takada, H. Fujita, Y. Yano, Y. Tsuboi, and T. Ikeda.** 1993. Identity of pathogenic strains of spotted fever rickettsiae isolated in Shikoku District based on reactivities to monoclonal antibodies. Jpn. J. Med. Sci. Biol. **46:**45–49.
13. **Parola, P., and D. Raoult.** 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. **32:**897–928.
14. **Raoult, D., and V. Roux.** 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin. Microbiol. Rev. **10:**694–719.
15. **Roux, V., P. E. Fournier, and D. Raoult.** 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR amplified DNA of the gene encoding the protein rOmpA. J. Clin. Microbiol. **34:**2058–2065.
16. **Roux, V., and D. Raoult.** 1995. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. Res. Microbiol. **146:**385–396.
17. **Roux, V., E. Rydkina, M. Eremeeva, and D. Raoult.** 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int. J. Syst. Bacteriol. **47:**252–261.
18. **Sekeyova, Z., P. E. Fournier, J. Rehacek, and D. Raoult.** 2000. Characterization of a new spotted fever group rickettsia detected in *Ixodes ricinus* (Acari: Ixodidae) collected in Slovakia. J. Med. Entomol. **37:**707–713.
19. **Takada, N.** 1995. Recent findings on vector acari for rickettsia and spirochete in Japan. Jpn. J. Sanit. Zool. **46:**91–108.
20. **Takada, N., H. Fujita, Y. Yano, Y. Tsuboi, and F. Mahara.** 1994. First isolation of a rickettsia closely related to Japanese spotted fever pathogen from a tick in Japan. J. Med. Entomol. **31:**183–185.
21. **Takada, N., F. Ishiguro, H. Iida, Y. Yano, and H. Fujita.** 1994. Prevalence of Lyme *Borrelia* in ticks, especially *Ixodes persulcatus* (Acari: Ixodidae), in central and western Japan. J. Med. Entomol. **31:**474–478.
22. **Uchida, T., T. Uchiyama, K. Kumano, and D. H. Walker.** 1992. *Rickettsia japonica* sp. nov., the etiological agent of spotted fever group rickettsiosis in Japan. Int. J. Syst. Bacteriol. **42:**303–305.
23. **Wood, D. O., L. R. Williamson, H. H. Winkler, and D. C. Krause.** 1987. Nucleotide sequence of the *Rickettsia prowazekii* citrate synthase gene. J. Bacteriol. **169:**3564–3572.
24. **Yano, Y., N. Takada, and H. Fujita.** 1993. Ultrastructure of spotted fever rickettsialike microorganisms observed in tissues of *Dermacentor taiwanensis* (Acari:Ixodidae). J. Med. Entomol. **30:**579–585.
25. **Yano, Y., N. Takada, and H. Fujita.** 2000. Ultrastructure of spotted fever group rickettsiae in tissues of larval *Amblyomma testudinarium* (Acari: Ixodidae). J. Acarol. Soc. Jpn. **9:**181–184.
26. **Zhang, J. Z., M. Y. Fan, Y. M. Wu, P. E. Fournier, V. Roux, and D. Raoult.** 2000. Genetic classification of *Rickettsia heilongjiangii* and *Rickettsia hulinii*, two Chinese spotted fever group rickettsiae. J. Clin. Microbiol. **38:**3498–3501.