were typical of lymphangitis-associated rickettsiosis, and most cases of rickettsioses in southern France in the spring are caused by *R. sibirica mongolitimonae*. Clustered cases of SFG rickettsiae infection have been reported in Europe, including southern France (*3*,*6*). In 2007, *R. conorii* and *R. massiliae* infections in humans were reported (*3*). In 2010, cases for which we were unable to discriminate between *R. conorii* and *R. massiliae* infections occurred in a family (*6*). In these 2 studies, clustered cases of SFG rickettsiosis involved *Rh. sanguineus* ticks. Clustered cases appeared to be related to an increase in aggressiveness of ticks toward humans during warmer periods (*3*). In our study, no correlation was identified with warmer weather.

R. sibirica mongolitimonae is most frequently associated with *Hyalomma* spp. ticks (*1*,*2*,*4*). However, 1 case of infection with this bacterium was associated with *Rh. pusillus* ticks collected in Portugal (*7*); DNA from this bacteria was also identified in an *Rh. pusillus* tick collected from a mongoose. The European wild rabbit is the primary host of *Rh. pusillus* ticks. However, these ticks have been found on wild carnivorous animals, dogs, and domestic cats (*8*); these ticks can bite humans (*8*). Moreover, *R. massiliae* and *R. sibirica mongolitimonae* were found in *Rh. pusillus* ticks from Spain (*9*), and SFG rickettsiae were found in ticks from Sardinia (*10*). Therefore, *Rh. pusillus* ticks appear to be an emerging vector for *R. sibirica mongolitimonae* in Europe.

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DOI: http://dx.doi.org/eid1902.120863

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Rickettsiae in Ticks, Japan, 2007–2011

To the Editor: Japanese spotted fever (JSF), caused by *Rickettsia japonica*, is the most prevalent tick-borne infectious disease in Japan (*1*), occurring most frequently in central and western regions (http://idsc.nih.go.jp/idwr/CDROM/Main.html [in Japanese]). Cases of unknown fever with rickettsiosis-like symptoms not associated with JSF have been reported in JSF-endemic regions of Japan (*2*). Several spotted fever group (SFG) rickettsiae (*R. japonica*, *R. heilongjiangensis*, *R. helvetica*, *R. tamurae*, *R. asiatica*, *Candidatus* R. tarasevichiae) and other related *Rickettsia* spp. have been identified in Japan (*1*,*3*–*6*). Human infections with *R. heilongjiangensis* and *R. tamurae* have been confirmed (*3*,*5*), and *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*, known human pathogens, have been detected in ticks and deer in Japan. We conducted this study to determine the risk in central and western Japan for human exposure to ticks harboring SFG rickettsiae, *A. phagocytophilum*, or *Ehrlichia* spp.

In 2007–2011, we collected 827 *Haemaphysalis*, *Amblyomma*, and *Ixodes* spp. ticks (392 adults, 435 nymphs) by flagging vegetation in the prefectures of Shizuoka, Mie, Wakayama, Kagoshima, Nagasaki (Goto Island), and Okinawa (the main island and Yonaguni Island) (Technical

Appendix Figure 1, wwwnc.cdc.gov/EID/article1/12-0856-Techapp/.pdf). We extracted DNA from the salivary glands of each tick and performed PCR to amplify *gltA*, 16S rDNA, and *ompA* of SFG rickettsiae. To detect *A. phagocytophilum* and *Ehrlichia* spp., we performed nested PCR targeting the *p44/msp2* and *p28/omp-1* multigenes, respectively.

PCR *gltA* screening revealed SFG rickettsiae in 181 (21.9%) of the 827 ticks (Table). We obtained nearly full-length (1.1-kb) *gltA* sequences and classified them into 5 groups by phylogenetic analyses (Technical Appendix Figure 2). Sequences for groups 1 (prevalence 1.0%) and 2 (prevalence 3.2%) were identified as *R. japonica* YH (GenBank accession no. AP011533) and *R. tamurae* (GenBank accession no. AF394896), respectively (Table). Group 3 (prevalence 15.1%) sequences were identical to that of *Rickettsia* sp. LON (GenBank accession no. AB516964). The sequence for group 4 (prevalence 1.6%) was closely related to that for *R. raoultii* strain Khabarovsk (98.8% similarity), and a part of the sequence (342 bp) was identical to that of *Rickettsia* sp. Hf 151 (GenBank accession no. AB114815). Group 5 consisted of 4 newly identified rickettsiae (Technical Appendix Figure 2). Of these 4 rickettsiae, 3 (Mie311, Goto13, and Mie334) were closely related to *R. raoultii* strain Khabarovsk (98.0% identity) and 1 (Mie201) was similar to *Candidatus* R. principis (99.7% identity).

We further analyzed the 16S rDNA and *ompA* in *gltA*-positive tick samples. The 16S rDNA and *ompA* for group 1 samples shared 100% identity with 16S rDNA and *ompA* of *R. japonica* YH (AP011533). The 16S rDNA of group 2 was identical to that of *R. tamurae* (AY049981). In groups 3–5, some of the specific amplicons in 16S rDNA or *ompA* could be detected; their sequences were confirmed to be similar (but not identical) to those of several known rickettsial sequences.

We amplified the *p44/msp2* amplicons of *A. phagocytophilum* from 25 (3%) of 827 ticks (Table). By cloning (TA Cloning Kit; Life Technologies, Carlsbad, CA, USA) and sequencing these amplicons, we obtained and identified 60 new TA-clone sequences (366–507 bp) for *p44/msp2* (GenBank accession nos. JQ697880–JQ697950); these sequences may include a potentially novel *Anaplasma* species. (*7*). *Ehrlichia p28/omp-1* was detected from 2 (0.2%) of the 827 ticks. Of 5 TA-clone sequences (284–315 bp) obtained from the 2 ticks, 2 from an *A. testudinarium* tick (GenBank accession nos. JQ697886 and JQ697887) shared 83.3%–86.7% similarity with *E. ruminantium* Gardel Map-1 (GenBank accession no. YP196842), and 3 from an *H. longicornis* tick (GenBank accession nos. JQ697888–JQ697890) showed the closest relationship to *E. ewingii omp-1–15* (67%–73% similarity; GenBank accession no. EF116932).

We identified the tick species associated with *R. japonica* as *H. formosensis*, *H. hystricis*, and *H. cornigera*, and another study reported an association with *Dermacentor taiwanensis*, *H. flava*, *H. longicornis*, and *I. ovatus* (*4*). In our study and previous studies, the tick species associated with *A. phagocytophilum* in Japan were identified as *H. formosensis*, *H. longicornis*, *H. megaspinosa*, *A. testudinarium*, *I. ovatus*, and *I. persulcatus* (*8*). Thus, it appears that 3 tick species (*H. formosensis*, *H. longicornis*, and *I.*

Table. PCR survey results for *Haemaphysalis*, *Amblyomma*, and *Ixodes* spp. ticks tested for rickettsiae, central and western Japan, 2007–2011*

Tick species	No. ticks tested	Total no. (%) ticks positive	No. (%) ticks positive for *Rickettsia gltA*, by species group†					*A. phagocytophilum p44/msp2*	*Ehrlichia p28/omp-1*§
			Group 1	Group 2	Group 3	Group 4	Group 5		
H. formosensis	224	6 (2.7)	1 (0.4)	0	0	0	5 (2.2)	18 (8)	0
H. hystricis	97	19 (19.6)	6 (6.1)	0	0	13 (13.4)	0	0	0
H. longicornis	294	119 (40.5)	0	0	119 (40.5)	0	0	2 (0.7)	1 (0.4)‡
H. flava	55	6 (10.9)	0	0	2 (3.6)	0	4 (7.3)	0	0
H. kitaokai	10	0	0	0	0	0	0	0	0
H. megaspinosa	18	4 (22.2)	0	0	4 (22.2)	0	0	1 (5.6)	0
H. cornigera	11	1 (9.1)	1 (9.1)	0	0	0	0	0	0
A. testudinarium	112	26 (23.2)	0	26 (23.2)	0	0	0	3 (2.7)	1 (0.9)
A. geoemydae	1	0	0	0	0	0	0	0	0
I. ovatus	5	0	0	0	0	0	0	1 (20.0)	0
Total	827	181 (21.9)	8 (1.0)	26 (3.1)	125 (15.1)	13 (1.6)	9 (1.1)	25 (3.0)	2 (0.2)

*DNA was extracted from the salivary glands of each tick by using the DNeasy Mini Kit (QIAGEN Sciences, Germantown, MD, USA) and used as a template for PCR. The newly identified sequences of *gltA,* 16S rDNA, *ompA*, *p44/msp2*, and *p28/omp*-1 in this study were deposited into GenBank under accession nos. JQ697880–JQ697959. *A. phagocytophilum, Anaplasma phagocytophilum.*

†The PCR primers used, *gltA*–Fc (5′-CGAACTTACCGCTATTAGAATG-3′) and *gltA*–Rc (5′-CTTTAAGAGCGATAGCTTCAAG-3′), were designed in this study. Groups: 1, *Rickettsia japonica* YH (GenBank accession no. AP011533); 2, *R. tamurae* (GenBank accession no. AF394896); 3, *Rickettsia* sp. LON-13 (GenBank accession no. AB516964); 4, *Rickettsia* sp. Hf151; 5, other rickettsiae.

‡PCR primers of p3726 (5′-GCTAAGGAGTTAGCTTATGA-3′), p3761 (5′-CTGCTCT[T/G]GCCAA(AG)ACCTC-3′, p4183 (5′-CAATAGT[C/T]TTAGCTAGTAACC-3′), and p4257 (5′-AGAAGATCATAACAAGCATTG-3′) were used for detection of *p44/msp2*.

§PCR primers conP28-F1 (5′-AT[C/T]AGTG[G/C]AAA[A/G]TA[T/C][A/G]T[G/A]CCAA-3′), conP28-F2 (5′-CAATGG[A/G][T/A]GG[T/C]CC[A/C]AGA[A/G]TAG-3′), conP28-R1 (5′-TTA[G/A]AA[A/G]G[C/T]AAA[C/T]CT[T/G]CCTCC-3′), and conP28-R2 (5′-TTCC[T/C]TG[A/G]TA[A/G]G[A/C]AA[T/G]TTTAGG-3′) were used to detect *p28/omp-1*.

ovatus) are associated with *R. japonica* and *A. phagocytophilum*.

In addition, in an *H. formosensis* tick, we detected an SFG rickettsia that is closely related to *R. raoultii*, the etiologic agent of *Dermacentor*-borne necrosis erythema and lymphadenopathy in Europe and Russia (*9*). We detected *Candidatus* R. principis in *H. flava* in Japan; this species was previously detected in *H. japonica douglasi* and *H. danieli* ticks in Russia and China, respectively, (*10*). And, we found a high prevalence of *R. tamurae* in *A. testidinarium* ticks; Imaoka et al. (*5*) recently reported that *R. tamurae* causes local skin inflammation without general JSP-like symptoms. We did not detect the human pathogen *E. chaffeensis*, but we identified 2 potentially new *Ehrlichia* species.

Our findings contribute to the known risks for exposure to *Rickettsia*-related pathogens in central and western Japan. Further studies may be required for the surveillance of additional pathogens, such as *Candidatus* Neoehrlichia mikurensis (*2*), which was recently recognized as a human pathogen.

This work was supported by the Research on Emerging and Reemerging Infectious Diseases grant from the Association for Preventive Medicine of Japan; grants for Research on Emerging and Reemerging Infectious Diseases from the Japanese Ministry of Health, Labor and Welfare (H18-Shinkou-Ippan-014, H21-Shinkou-Ippan-006, and H24-Shinkou-Ippan-008); and a Global Center of Excellence Program grant from Japanese Ministry of Education, Culture, Sports, Science and Technology (to N.O.).

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DOI: http://dx.doi.org/10.3201/eid1902.120856

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