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***Rickettsia* sp. in *Ixodes granulatus* Ticks, Japan**

To the Editor: The genus *Rickettsia* consists of obligate intracellular bacteria that cause spotted fever and typhus fever; these bacteria are usually transmitted by an arthropod vector. We report isolation of a *Rickettsia honei*-like organism from the *Ixodes granulatus* tick; this organism may be a causative agent of rickettsiosis in Japan. Serotyping and DNA-sequencing analysis distinguished this *I. granulatus* isolate from previously reported *Rickettsia* spp.

During 2004–2005, an investigation of rickettsiosis was conducted in Okinawa Prefecture in the southernmost part of Japan, an area known to be inhabited by *I. granulatus*, a parasitic tick commonly found on small mammals. A total of 43 *I. granulatus* ticks (3 larvae, 27 nymphs, 8 adult females, and 5 adult males) were collected from small mammals (*Rattus rattus*, *R. norvegicus*, *Suncus murinus*, *Mus calori*, and *Crocidura watasei*) for the present study. For the isolation of *Rickettsia* spp., the cell line L929 was used as previously described (1). A total of 13 isolates, designated as strains GRA-1 to GRA-13, were obtained from 11 ticks (1 fed larva, 5 fed nymph, 1 fed adult female, 1 fed adult male, 1 unfed nymph molted from engorged larva, 2 unfed adult females molted from engorged nymphs) and from 1 pool of eggs and 1 larva derived from the engorged female tick.

Serotyping was performed by using a microimmunoperoxidase approach according to the method described by Philip et al. (2); we used anti-*Rickettsia* mouse serum and several spotted fever group *Rickettsia* antigens: 2 of the present isolates (GRA-1 and GRA-2) and 6 known members of the Asian *Rickettsia* spp. (*R. honei*, *R. japonica*, *R. asiatica*, *R. tamurae*, *R. sibirica*, and *R. conorii*). Differences among antigen reaction titers were calculated, and the results are given as the specificity difference (SPD) value. The SPD value between the present isolates and *R. honei* was 0 or 1, whereas the SPD values were ≥ 3 for the other spotted fever group *Rickettsia* spp. (Table). According to the criteria for serotyping (2), we assumed the isolates to be of the same serotype when the SPD value was ≤ 2 . In addition to serotyping, a sequencing analysis was performed to genetically characterize the isolates. The archive of DNA sequences has been mostly established for the outer membrane protein A gene (*ompA*), citrate synthesis gene (*gltA*), and 17-kDa antigen gene. Thus, we determined these DNA sequences in the isolates and compared the results with those of representative *Rickettsia* spp. The *ompA* sequencing analysis showed a DNA sequence of 491 bp in the 6 isolates from *I. granulatus* (GenBank accession nos. AB444090–AB444095), which yielded the following similarity values: *R. slovacica* (98.0%), *R. honei* and Thai tick typhus *Rickettsia* (97.8%), and *R. honei* subsp. *marmionii* (97.6%). Sequencing of the 1,250-bp fragment of *gltA* of the strain GRA-1 (accession no. AB444098) showed >99% DNA similarity with that of *R. sibirica* (99.3%), *R. slovacica* (99.2%), *R. conorii* (99.2%), *R. honei* (99.1%), and certain types of *Rickettsia* spp. Moreover, 17-kDa antigen gene sequencing analysis of a 392-bp fragment of the strain GRA-1 (accession no. AB444097) showed the highest levels of sequencing similar-

Table. Serotype results for *Rickettsia* sp. strains GRA-1 and GRA-2 from *Ixodes granulatus* tick, Okinawa Prefecture, Japan

Mouse antiserum to	Results*							
	GRA-1	GRA-2	TT-118	Aoki	IO-1	AT-1	246	Moroccan
Strains from this study								
<i>Rickettsia</i> sp., GRA-1	320 †	320 (0)‡	160 (1)	80 (3)	40 (6)	40 (6)	80 (4)	80 (3)
<i>Rickettsia</i> sp., GRA-2	320 (0)	320	320 (0)	40 (4)	20 (7)	40 (7)	40 (5)	40 (5)
Reference strains								
<i>R. honei</i> , TT-118	5,120 (1)	5,120 (0)	5,120	80 (7)	320 (7)	80 (9)	160 (7)	80 (7)
<i>R. japonica</i> , Aoki	2,560 (3)	2,560 (4)	1,280 (7)	5,120	320 (9)	320 (10)	320 (9)	320 (8)
<i>R. asiatica</i> , IO-1	640 (6)	640 (7)	80 (7)	160 (9)	5,120	80 (12)	80 (11)	160 (11)
<i>R. tamurae</i> , AT-1	640 (6)	320 (7)	80 (9)	80 (10)	80 (12)	5,120	160 (11)	40 (13)
<i>R. sibirica</i> , 246	1,280 (4)	1,280 (5)	320 (7)	160 (9)	160 (11)	80 (11)	5,120	320 (7)
<i>R. conorii</i> , Moroccan	640 (3)	640 (5)	80 (7)	80 (8)	20 (11)	20 (13)	160 (7)	1,280

*Highest serum dilutions against each *Rickettsia* antigen (specificity difference between each pair of strains), determined by microimmunoperoxidase method. **Boldface** indicates equivocal titer to homologous antigen.

†Highest serum dilution showing a positive reaction with antigen.

‡Specificity difference between each pair of strains.

ity value with *R. honei* and Thai tick typhus *Rickettsia* (99.5%) compared with those of the sequences of other deposited *Rickettsia* spp. Comprehensive analyses led us to presume that the isolate GRA-1 from *I. granulatus* was a genetic variant of *R. honei*, although further studies are necessary to better define the taxonomic position of our isolates.

The vector for *R. honei* was presumed to be ixodid ticks: *I. granulatus* in Thailand; *Amblyomma cajennense* in Texas, USA; and *Aponomma hydrosauri* in Australia (3–5). Lane et al. reported that a *Rickettsia* organism from a *Haemaphysalis* tick was closely related to *R. honei* in Australia (6). In the present study, we observed that the *Rickettsia* organism was maintained in the tick after molting. Moreover, *Rickettsia* organisms were also isolated from egg and unfed larva. These preliminary findings may suggest that *I. granulatus* is a possible vector for the *R. honei*-like bacterium in Japan.

Recently, a *Rickettsia* sp. was found in *I. granulatus* ticks; its proposed designation was unclassified *Rickettsia* IG-1, according to DNA sequencing from specimens obtained in Taiwan (7). With respect to the DNA sequences of *gltA* and *ompA*, our isolates from *I. granulatus* were identical to the *Rickettsia* IG-1.

R. honei, a member of the spotted fever group *Rickettsia*, has been reported as the etiologic agent of

Flinders Island spotted fever in Australia (8) and also of Thai tick typhus (3). *R. honei* is a public health threat for rickettsiosis in these countries. Although the human health implications of the *Rickettsia* sp. found in this study are not yet known, knowledge from this study will be useful in epidemiologic investigation for rickettsiosis in Japan.

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Sin Nombre Virus Infection in Deer Mice, Channel Islands, California

To the Editor: Sin Nombre virus (SNV) is a highly virulent strain of hantavirus associated with rodent hosts in North America (1,2). Documenting the prevalence of SNV in wild rodent populations is an important component of determining risk for exposure and ultimately providing sound recommendations for epidemiologic management (3). Prevalence of SNV is highly variable. Deer mice (*Peromyscus maniculatus*) that inhabit the Channel Islands off the California coast often have rates of SNV that greatly exceed values on the mainland (2). Even though these islands have high rates of SNV prevalence and are recreational areas for humans, no surveys of the Channel Islands have been performed to document the dynamics of prevalence since 1994–1996 (2,4). We visited 4 of the Channel Islands in 2007 to document rates of SNV prevalence in *P. maniculatus*.

From May 3–15, 2007, we visited 4 of the Channel Islands off the California coast: East Anacapa Island, Santa Barbara Island, San Miguel Island, and Santa Rosa Island. On each island, mice were captured by using Sherman live traps from habitats characterized by giant coreopsis (*Coreopsis gigantea*), a shrub native to California, to provide a standardized habitat for comparisons across islands. The number of sampling areas

depended largely upon the distribution of *C. gigantea* habitat and logistical considerations during each island visit (Table). Upon capture of the mice, blood samples were taken from the submandibular vein by using Medi-Point animal lancets (Medi-Point International, Inc., Mineola, NY, USA) and stored in sterile micropipette tubes. Samples were stored on ice until shipment to the California Department of Health Services' Viral and Rickettsial Disease Laboratory for processing. *P. maniculatus* serum samples were examined for immunoglobulin (Ig) G antibodies to the SNV nucleocapsid protein by ELISA with Centers for Disease Control and Prevention reagents (5).

Detailed information regarding SNV prevalence, sampling location, and sampling effort is presented in the Table. We compare our 2007 data with data collected in 1994 by Jay et al. (2) because 1994 was the only other year when all 4 islands used in our study were sampled. Graham and Chomel (4) also collected data from San Miguel Island and Santa Rosa Island in 1995 and 1996 (the use of the average prevalence from 1995 and 1996 for these 2 islands does not change any of our results).

There was no significant difference in prevalence of SNV antibodies between our 2007 results and the prevalence found by Jay et al. (2) in 1993–1994 (paired *t* test $t = 0.13$, 3; $df = 3$; $p = 0.91$). Overall, 36 male and 42 female mice were captured; the sex of

captured animals was independent of SNV infection (9 males and 6 females positive for SNV; test of independence $\chi^2 = 0.28$, 1 *df*, $p = 0.59$). We captured only 2 subadult mice on islands where we also detected antibodies to SNV; 1 mouse tested positive, the other tested negative. Although our sample sizes precluded detecting very low rates of SNV infection with confidence on Santa Barbara and East Anacapa Islands, the consistency of our results with those of Jay et al. (2) suggests that our sampling was sufficient for comparative purposes.

Several studies now indicate the importance of long-term surveillance of SNV prevalence in wild rodent populations for understanding the factors that may contribute to outbreaks of human disease, e.g. (6). These studies often document the generally positive, though often temporally delayed, relationship between population density of *P. maniculatus* and seroprevalence for SNV (7). Our results suggest a high degree of temporal stability in prevalence of antibodies to SNV in *P. maniculatus* on the Channel Islands, despite considerable variation in host population density between earlier studies and ours (4,8). Although we cannot know the prevalence of SNV among *P. maniculatus* on the Channel Islands in periods between the studies by Jay et al. (2), Graham and Chomel (4), and our own, SNV prevalence on these islands is quite similar to levels previously recorded both for islands with relatively low prevalence

Table. Sin Nombre virus in *Peromyscus maniculatus* mice on 4 Channel Islands, California, May 3–15, 2007*

Island*	No. trap nights	Prevalence, %	
		2007	1994
East Anacapa†	180	0	0
San Miguel‡	104	26.3	17.9
Santa Barbara§	216	0	0
Santa Rosa¶	216	47.6	58

*The number of captured mice that were sampled for Sin Nombre virus (SNV) was 23 on East Anacapa, 19 on San Miguel Island, 15 on Santa Barbara Island, and 21 on Santa Rosa Island. The 1994 data in the table are from a study by Jay et al. (2) and are included for comparison purposes.

†East Anacapa: 34°00'56"N/119°21'49"W.

‡San Miguel: 34°02'18"N/120°20'54"W.

§Santa Barbara: 33°28'30"N/119°02'12"W.

¶Santa Rosa: 34°00'03"N/120°03'30"W.