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Rickettsia raoultii–like Bacteria in *Dermacentor* spp. Ticks, Tibet, China

To the Editor: *Rickettsia raoultii* is an obligate intracellular gram-negative bacterium belonging to the spotted fever group (SFG) of the genus *Rickettsia*. Genotypes RpA4, DnS14, and DnS28, originally isolated from ticks from Russia in 1999 (*1*), were designated as *Rickettsia raoultii* sp. nov. on the basis of phylogenetic analysis (*2*). *R. raoultii* has been found mainly in *Dermacentor* spp. ticks in several countries in Europe (*3*). It was detected in a *Dermacentor marginatus* tick from the scalp of a patient with tick-borne lymphadenitis in France (*2*), which suggests that it might be a zoonotic pathogen. We determined the prevalence of *R. raoultii*–like bacteria in *Dermacentor* spp. in highland regions in Tibet.

Ticks from sheep (*Ovis aries*) near Namuco Lake (a popular tourist destination 4,718 m above sea level) were collected and identified morphologically as *D. everestianus* and *D. niveus* ticks (*4*). Genomic DNA was extracted from individual specimens by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). All DNA samples were amplified by using PCRs specific for the citrate synthase (*gltA*, 770 bp) gene (*5*) and the outer membrane protein A (*ompA*, 629 bp) gene (*6*). Some samples were amplified by using a PCR specific for the *ompB* (2,479 bp) gene (*7*).

Randomly selected amplicons for *gltA* (n = 27), *ompA* (n = 31), and *ompB* (n = 7) were cloned into the pGEM-T Easy vector (Promega, Shanghai, China) and subjected to bidirectional sequencing (Sangon Biotech, Shanghai, China). Sequences obtained were deposited in GenBank under accession nos. JQ792101–JQ792105, JQ792107, and JQ792108–JQ792166. Phylogenetic analysis was conducted for sequences we identified and sequences of recognized SFG rickettsial species available in Genbank by using the MegAlign program (DNASTAR, Inc., Madison, WI, USA) and MEGA 4.0 (*8*).

Of 874 tick specimens, 86 were *D. everestianus* ticks (13 male and 73 female), and 788 were *D. niveus* ticks (133 male and 655 female). Samples positive for *gltA* and *ompA* were considered SFG rickettsial species. Using this criterion, we found that 739 tick specimens (84.6%) were positive for *Rickettsia* spp. Of 86 *D. everestianus* ticks, 85 (98.8%) were positive for *Rickettsia* spp. and of 788 *D. niveus* ticks, 654 (83.0%) were positive. Infection rates for male and female *D. niveus* ticks were 87.9% and 82.1%, respectively. We found an overall prevalence of 84.6% for *R. raoultii*–like bacteria in *Dermacentor* spp. in the highland regions in Tibet.

Nucleotide sequence identities ranged from 99.2% to 100% (except for isolate WYG55, which had an identity of 98.6%) for the *ompA* gene and from 99.2% to 99.9% (except for isolate XG86, which had an identity of 98.5%) for the *ompB* gene. These results indicated that homology levels of most isolates were within species thresholds (*ompA* ≈98.8% and *ompB* ≈99.2%) (*9*). Isolate WYG55 showed the lowest identity (98.2%) among *gltA* gene sequences and the lowest identity (98.6%) among *ompA* gene sequences. Isolate XG86 showed lowest identity (98.5%) among *ompB* gene sequences. These results suggest that other *Rickettsia* spp. were among the investigated samples.

A BLASTn search (www.ncbi.nlm.nih.gov/) for the obtained sequences was conducted. The best matches (highest identities) detected were with sequences of *R. raoultii*. However, comparison of our sequences with corresponding sequences of *R. raoultii* in GenBank showed identity ranging from 98.0% to 99.0% for

ompA and from 98.1% to 99.0% for *ompB*, which did not meet the threshold (*9*) for *R. raoultii*. We compared the new sequences with corresponding reference sequences of universally recognized SFG group *Rickettsia* spp. in Genbank and constructed 2 phylogenetic trees (Figure). The new sequences were placed into separate branches, which were closely related to *R. raoultii* branches.

Prevalence of *R. slovaca* and *R. raoultii* was 6.5% and 4.5% in *D. silvarum* ticks in Xinjiang Uygur Autonomous Region of China (*10*). In contrast, we found that the overall prevalence of *R. raoultii*–like bacteria might be ≤84.6% in *D. everestianus* and *D. niveus* ticks in Dangxiong County in Tibet.

Our findings suggest that *D. everestianus* and *D. niveus* ticks are potential vectors of *R. raoultii*–like bacteria and indicate that spread of *R. raoultii*-like bacteria encompasses a large area in China. In the study sites, yak and Tibetan sheep are the major domestic animals, and rodents are the major wild animals. Rodents are also the major hosts of *Dermacentor* spp. ticks, which can transmit *R. raoultii* transstadially and transovarially (*2*). Animals bitten by infected ticks can acquire the pathogen and serve as natural reservoirs.

On the basis of phylogenetic analysis, we found that the *Rickettsia* spp. in ticks investigated represents a novel species, which can be designated *Candidatus* Rickettsia tibetani. However, additional phylogenetic studies are needed to obtain more information on the molecular biology of these bacteria.

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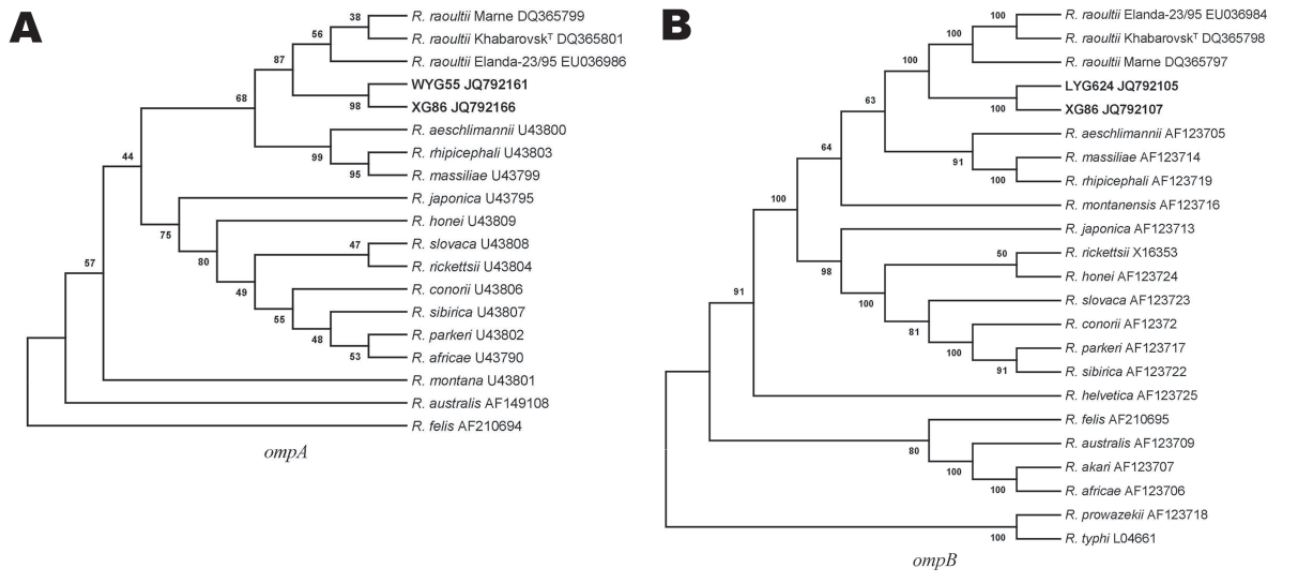

Figure. Unrooted phylogenetic trees inferred from comparison of A) outer membrane protein A (*ompA*) and B) *ompB* gene sequences of rickettsial species by using the neighbor-joining method. Sequences in **boldface** were obtained during this study. Numbers at nodes are the proportion of 100 bootstrap resamplings that support the topology shown.

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Leishmania (*Viannia*) *guyanensis* Infection, Austria

To the Editor: Infection with *Leishmania* spp. was diagnosed in an asymptomatic soldier during an explorative national cross-sectional serologic screening of soldiers volunteering for United Nations missions at the Military Hospital Vienna in 2009. Diagnosis was made by using a commercial ELISA kit (Ridascreen *Leishmania*; R-Biopharm, Darmstadt, Germany). One year later, the soldier was reassessed for persisting antibodies by using the same ELISA and for *Leishmania* DNA in a blood sample stored in EDTA by using the *Leishmania* OligoC-Test (Coris BioConcept, Gembloux, Belgium). (The study was approved by the Research Ethics Committee of the Austrian Armed Forces and written informed consent was obtained from the person investigated.) Because the results of both tests were positive, an additional PCR was performed for identification below genus level with the LITSR/L5.8S primer pair (*1*). To confirm the PCR results, we sequenced the amplicon in both directions in 2 independent setups and compared the obtained 299-bp sequence with published sequences from GenBank by performing a multiple sequence alignment. Our sequence (strain EN10) showed 100% (299/299 bp) identity with several strains from the *Leishmania (Viannia) guyanensis* complex, including the *L. guyanensis* strain MHOM/SR/87/TRUUS4 and the *L. panamensis* strains FJ948438, FJ948439, and FJ948446. Sequence homology to representatives of the *L. (V.) braziliensis* complex was ≈93%; to representatives of the *L. (Leishmania) mexicana* complex, 61%–68%; and to the *L. (L.) donovani* complex, 70%–71%. The *L. (V.) guyanensis* complex traditionally includes the species *L. guyanensis*, *L. panamensis,* and *L. shawi*, but *L. panamensis* seems to be a subspecies or even a synonym of *L. guyanensis* (*2*). We thus classified our strain as *L. guyanensis*. Sequence data were deposited at GenBank (accession no. JN671917).

L. guyanesis/panamensis is found in 9 countries in Central and South America (*3*). It is a common cause of zoonotic cutaneous leishmaniasis in humans. The sloths *Choloepus didactylus* (*L. guyanensis*) and *C. hoffmanni* (*L. panamensis*) are believed to be the principal reservoir hosts and the sandfly species *Lutzomyia umbratilis* (*L. guyanensis*) and *Lu. trapidoi* (*L. panamensis*) to be the principal vectors (*3*). Also, dogs can act as reservoirs for the *L. (V.) guyanensis* complex (*4*).

The infected soldier had never been to Central or South America and had no history of blood transfusions. His lifetime travel history included Italy, Spain, Greece, Germany, Croatia, New York City, and military assignments in Kosovo. Thus, how and where the infection had been acquired remain open for discussion.

Although sandflies are not as robust as *Anopheles* spp., for example, the most plausible scenario is that either an *L. guyanensis*–infected sandfly or a noninfected but transmissive sandfly from a disease-endemic area was transported in a ship or airplane (comparable to the well-known "airport malaria" situation) to an area where the patient had traveled. In recent years, *Lu. vexator* has become widespread and abundant in upstate New York (*5*). Although, this is not a known vector for *L. guyanensis*, its spread in New York State shows that *Lutzomyia* spp. can rapidly adapt to new and distant areas where the infection was previously nonendemic. Of the areas where the infected person had traveled, at least in New York City and Spain, regular introduction of *L. guyanensis* by immigrants, travelers, or dogs from Central and South