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Spotted Fever Group *Rickettsiae* in Inner Mongolia, China, 2015–2016

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We found *Rickettsia raoultii* infection in 6/261 brucellosis-negative patients with fever of unknown origin in brucellosis-endemic Inner Mongolia, China. We further identified *Hyalomma asiaticum* ticks associated with *R. raoultii*, *H. marginatum* ticks associated with *R. aeschlimannii*, and *Dermacentor nuttalli* ticks associated with both rickettsiae species in the autonomous region.

Spotted fever group rickettsiae (SFGR) are vector-borne pathogens. In China, 5 SFGR genotypes have been identified as causative agents of human rickettsiosis: *R. heilongjiangensis*, *R. sibirica* subsp. *sibirica* BJ-90,

Candidatus Rickettsia tarasevichiae, *R. raoultii*, and *Rickettsia* sp. XY99 (1–4).

Brucellosis, a zoonotic disease, is highly endemic to Inner Mongolia, China, and is increasing in workers in agriculture or animal husbandry (5). However, some agriculture workers with brucellosis-like symptoms, including general malaise and fever, were seronegative for *Brucella* spp. We suspected that fever of unknown origin among brucellosis-seronegative patients might be caused by tick-borne pathogens. We identified 6 cases of human *R. raoultii* infections in brucellosis-seronegative patients in western Inner Mongolia, and we investigated exposure to ticks infected with SFGR.

During 2015–2016, we obtained 261 blood samples from brucellosis-seronegative patients with fever of unknown origin in Bayan Nur Centers for Disease Control and Prevention (Bayan Nur City, Inner Mongolia, China). The review board of the Department of Medicine at College of Hetao (Bayan Nur City) approved the study. We extracted DNA from each blood sample using the DNeasy Mini Kit (QIAGEN, Hilden, Germany) and conducted PCR targeting SFGR *gltA* (6). The PCR primers used, *gltA*-Fc (5'-CGAACTTACCGCTATTAGAATG-3') and *gltA*-Rc (5'-CTTTAAGAGCGATAGCTTCAAG-3'), were described previously (4). We designed the primers 16S rDNA R-2F (5'-GAAGATTCTCTTTCCGGTTTCGC-3'), 16S rDNA R-2R (5'-GTCTTGCTTCCCTCTGTAAAC-3'), *ompA*-Fb (5'-GGTGCGAATATAGACCCTGA-3'), and *ompA*-Ra (5'-TTAGCTTCAGAGCCTGACCA-3') for this study and deposited the sequences obtained of *gltA*, *ompA*, and 16S rDNA into GenBank (accession nos. MH267733–47). We used genomic DNA extracted from L929 cells infected with *Rickettsia* sp. LON-13 (*gltA*: AB516964) as a positive control.

We detected *gltA* amplicons from 6/261 (2.3%) blood samples (Table). All 6 patients had strong malaise and mild fever of 36.8°C–37.3°C but no rash. Five of these patients also had arthralgia and vomiting.

Sequence and phylogenetic analysis showed that the sequences of 6 nearly full-length (1.1 kb) *gltA* amplicons with were identical to each other and to *R. raoultii* *gltA* (GenBank accession no. DQ365803). We further analyzed *ompA* and 16S rDNA in *gltA*-positive samples. All 6 samples were PCR positive for both genes; 552-bp sequences of the amplicons were identical to sequences of *R. raoultii* *ompA* (GenBank accession no. AH015610), and 389-bp sequences of the amplicons were identical to sequences of *R. raoultii* 16S rDNA (GenBank accession no. EU036982). PCR results were negative for the genes *Anaplasma phagocytophilum* *p44/msp2*, *Ehrlichia chaffeensis* *p28/omp-1*, and *Borrelia* spp. *flaB*. An indirect immunofluorescence assay showed that IgM and IgG titers against *R. japonica* were 40–80 for IgM in 3 patients and 160 for IgG in 2 patients.

Table. PCR survey of SFGR infections in patients and ticks, Inner Mongolia, China, 2015–2016*

Patient type or tick species	No. tested	No. (%) <i>gltA</i> positive for SFGR		
		<i>Rickettsia raoultii</i>	<i>R. aeschlimannii</i>	Total
Brucellosis-seronegative patients	261	6 (2.3)	0	6 (2.3)
Ticks				
<i>Hyalomma asiaticum</i>	766	118 (15.4)	0	118 (15.4)
<i>Hyalomma marginatum</i>	198	0	160 (80.8)	160 (80.8)
<i>Dermacentor nuttalli</i>	1,418	830 (58.5)	158 (11.1)	988 (69.7)†
<i>Rhipicephalus turanicus</i>	76	0	0	0
Total ticks	2,458	948 (38.6)	318 (12.9)	1,266 (51.5)

*SFGR, spotted fever group rickettsiae.

†We did not detect dual infection with *R. raoultii* and *R. aeschlimannii* in *D. nuttalli* ticks in this study.

To assess patients' risk of infection with SFGR by tick exposure, we collected 2,458 ticks morphologically identified as *Hyalomma marginatum* (n = 198), *H. asiaticum* (n = 766), *Dermacentor nuttalli* (n = 1,418), and *Rhipicephalus turanicus* (n = 76) from livestock and pet animals including sheep, cattle, camels, and dogs in western Inner Mongolia during 2015–2016 (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/11/16-2094-Techapp1.pdf>). We collected unattached ticks within animal hair, but not attached ticks. We prepared DNA extracted from salivary glands of each tick and conducted PCR screening by rickettsial *gltA* detection as described. We detected *gltA* in 1,266 (51.5%) of the total 2,458 ticks.

We classified the amplicons into 2 groups by restriction fragment-length polymorphism using *AluI* and *RsaI*, and we sequenced 25–45 representative amplicons in each group. On the basis of this analysis, we found that the sequences from the 2 groups were either identical to that of *R. raoultii* (GenBank accession no. DQ365803) or to that of *R. aeschlimannii* (GenBank accession no. HM050276) (Table; online Technical Appendix Figure 2). We detected *R. raoultii* DNA in *H. asiaticum* (118/766, 15.4%) and *D. nuttalli* (830/1,418, 58.5%) ticks and *R. aeschlimannii* DNA from *H. marginatum* (160/198, 80.8%) and *D. nuttalli* (158/1,418, 11.1%) ticks. We did not detect rickettsial DNA in *R. turanicus* ticks (0/76, 0%).

Recently, human cases of *R. raoultii* infection have been reported in China, including northeastern Inner Mongolia (1,4). Potential vectors for *R. raoultii* are *Dermacentor* spp. ticks in Europe, Turkey, and northern Asia and *Haemaphysalis* spp. and *Amblyomma* sp. ticks in southern Asia (7,8). Other studies have identified *Hyalomma* spp., *Rhipicephalus* spp., and *Amblyomma* sp. ticks as potential vectors for *R. aeschlimannii* (7,8); human cases of *R. aeschlimannii* infection have been reported in Italy and Morocco (7,9). We detected *R. raoultii* in *H. asiaticum* as well as *D. nuttalli* ticks, but in Mongolia, *R. raoultii* has been detected only in *D. nuttalli* ticks, and not *H. asiaticum* ticks (10). We identified *D. nuttalli* ticks as another potential vector for *R. aeschlimannii*. Our work contributes to the knowledge of the epidemiology, clinical characteristics, and known tick vectors associated with *R. raoultii* and *R. aeschlimannii*.

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Japanese Spotted Fever in Eastern China, 2013

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We isolated *Rickettsia japonica* from a febrile patient in Lu'an City, China, in 2013. Subsequently, we found an *R. japonica* seroprevalence of 54.8% (494/902) in the rural population of Anhui Province and an *R. japonica* prevalence in *Haemaphysalis longicornis* ticks of 0.5% (5/935). *R. japonica* and its tick vector exist in China.

¹These authors contributed equally to this article.

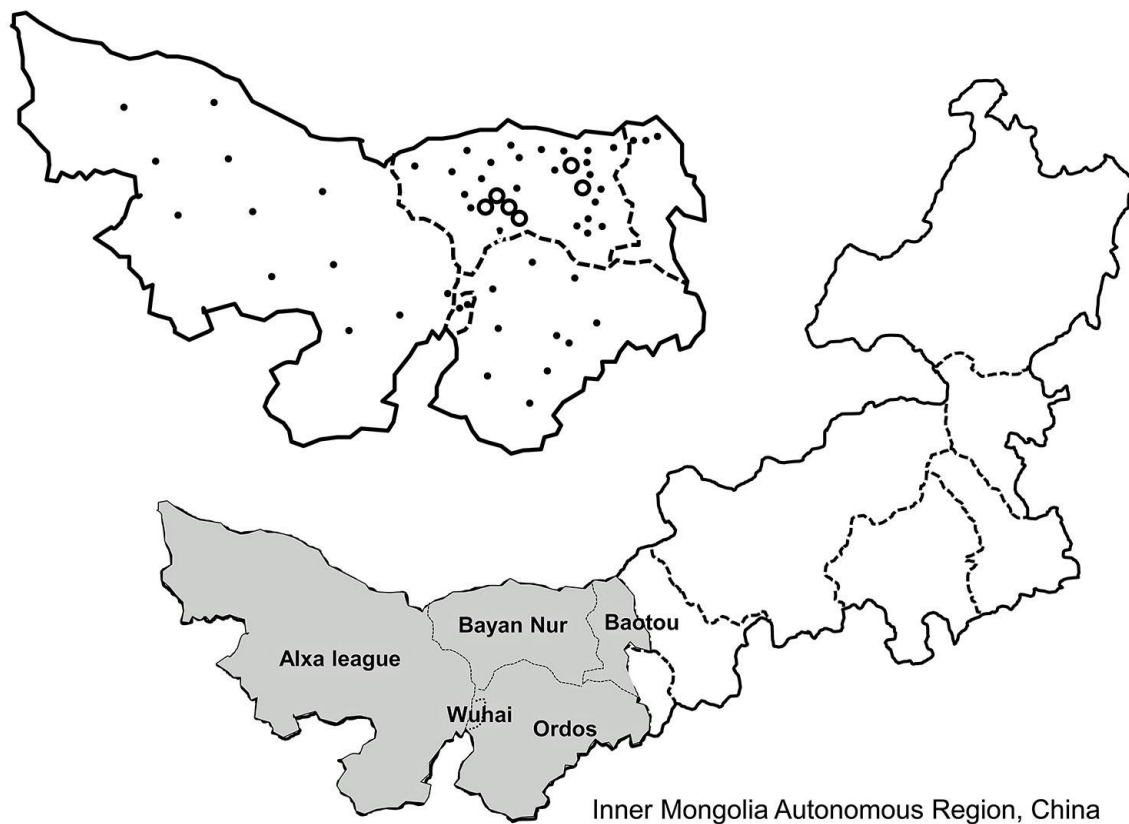
Spotted fever group rickettsiae are tickborne, obligatory intracellular, gram-negative bacteria with a worldwide distribution. However, the distribution of each species of spotted fever group rickettsiae is limited to geographic areas by their specific tick vectors. Japanese spotted fever is a severe rickettsiosis caused by *Rickettsia japonica* bacterium (1,2), which has been present in Japan since 1984 and isolated from patients in other countries of Asia (e.g., South Korea, the Philippines, and Thailand) over the past decade (3,4). In this study, we present information on an *R. japonica* isolate acquired from a febrile patient and *R. japonica* seroprevalence in Anhui Province in eastern China.

On August 7, 2013, a 61-year-old man from Shucheng County, Lu'an City, China, in the Dabie Mountain area of Anhui Province (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/11/17-0264-Techapp1.pdf>) with fever and headache for 1 week was admitted into Shucheng County People's Hospital. The patient reported several tick bites 10 days before the onset of his illness. At admission, the patient was conscious and had fever (39.0°C); he did not have jaundice, and no bleeding was found on his skin or mucosal membranes. A papular rash with papules 0.1–0.5 cm in diameter was noted all over his body (online Technical Appendix Figure 2). Blood cell counts showed the patient had leukocytosis (10.34×10^9 cells/L), increased neutrophils (87.5%), and a platelet count within reference range (130×10^9 /L). Blood chemistry testing revealed a urea nitrogen concentration of 9.12 mmol/L (reference range 2.9–8.2 mmol/L), creatinine of 0.758 mg/dL (67 μ mol/L, reference range 53–106 μ mol/L), C-reactive protein of 77.5 nmol/L (reference range 0.76–28.5 nmol/L), and an erythrocyte sedimentation rate of 22 mm/h (reference range 0–20 mm/h). A urine test showed a procalcitonin concentration of 0.806 ng/mL (reference range ≤ 0.15 ng/mL) and an interleukin 6 concentration of 52 pg/mL (reference range ≤ 1.8 pg/mL). The patient had rough lung breath sounds, and computed tomography showed inflammatory infiltrates in the middle right lung and lower left lung lobe, bullae on the upper left lung lobe, and emphysematous changes. The patient was suspected to have a rickettsial infection and was given minocycline and meropenem on the day of his admission. Two days later, on August 9, 2013, the patient's fever subsided (36.2°C), and he was discharged.

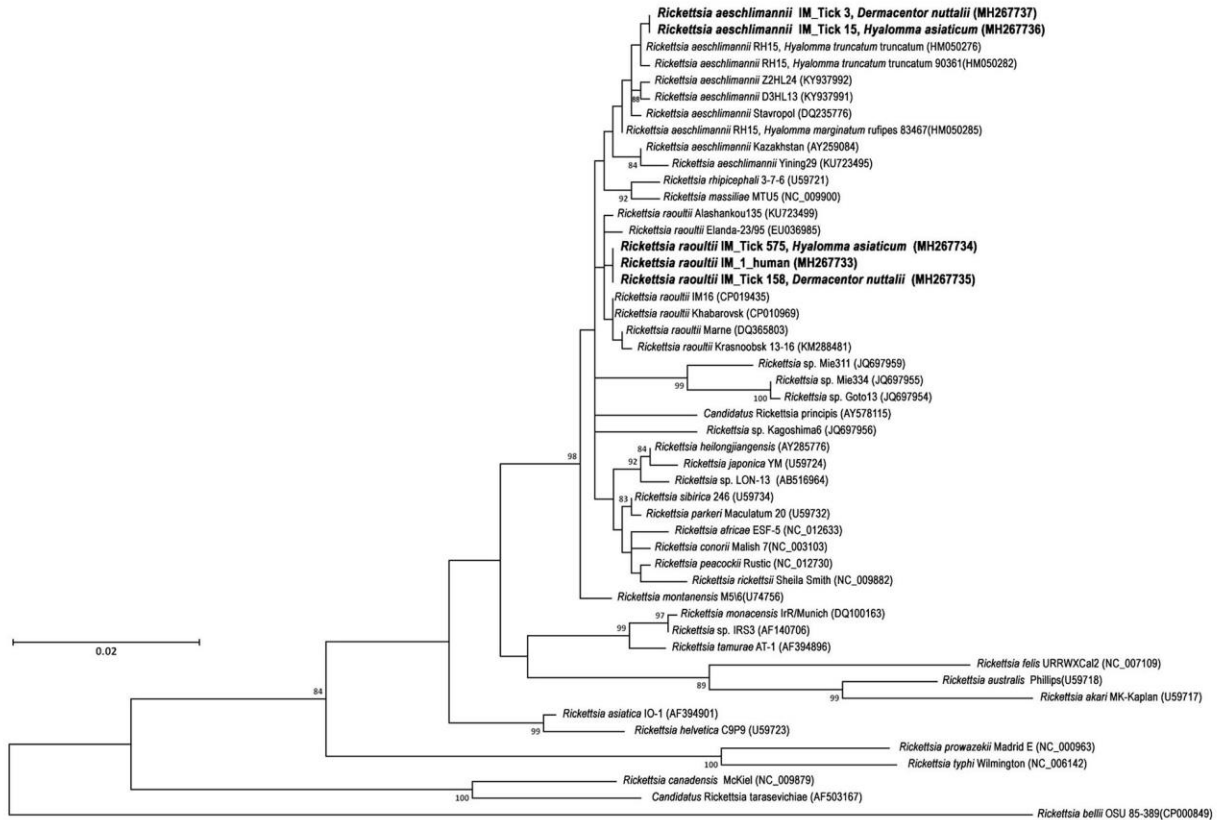
A blood sample taken from the patient 1 day after admission was inoculated onto THP-1 and Vero E6 cells; after 10 days, cytopathic effect was visible by light microscopy with only the THP-1 cells. Diff-Quick (Thermo Fisher Scientific, Kalamazoo, MI, USA)–stained smears of THP-1 cells showed *Rickettsia*-like bacilli in the cytoplasm. Electron microscopy showed the bacilli localized to the cytoplasm and nucleus and had the typical ultrastructure of *Rickettsia* bacteria. This species was highly pleomorphic

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Technical Appendix



Technical Appendix Figure 1. Locations of 6 patients with *Rickettsia raoultii* infection (open circles) and tick collection sites (solid dots) in western Inner Mongolia. Tick species collected were morphologically identified as *Hyalomma marginatum*, *H. asiaticum*, *Dermacentor nuttalli*, and *Rhipicephalus turanicus* that are known to be distributed in Asia, including Mongolia, and Turkey (1–3).



Technical Appendix Figure 2. Phylogenetic tree for spotted fever group rickettsiae based on *gItA* sequences (1,017 bp) in patients and in ticks in western Inner Mongolia, China. The tree was constructed using the maximum-likelihood method with 100 bootstrap resamplings in MEGA 6 (www.megasoftware.net). Bold indicates spotted fever group Rickettsia detected in this study. Scale bar indicates evolutionary distance.

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