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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 DNaseasy Mini Kit (QIAGEN, Hilden, Germany) and conducted PCR targeting SFGR gltA (6). The PCR primers used, gltA-Fc (5′-CGAACCTACGGCTATAGATG-3′) and gltA-Rc (5′-CTTTAAGAGCCATAGCTTCAAG-3′), were described previously (4). We designed the primers 16S rDNA R-2F (5′-GAAGATTTCTTCTCGTTTCCGC-3′), 16S rDNA R-2R (5′-GTCTTGCTTCCCTCTGTAAC-3′), rompA-Fb (5′-GGTGCGAATATAGACCCTGA-3′), and rompA-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 gltA, ompA, and 16S rDNA from brucellosis-seronegative patients with fever of unknown origin in Bayan Nur City, Inner Mongolia, China, 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 DNaseasy Mini Kit (QIAGEN, Hilden, Germany) and conducted PCR targeting SFGR gltA (6). The PCR primers used, gltA-Fc (5′-CGAACCTACGGCTATAGATG-3′) and gltA-Rc (5′-CTTTAAGAGCCATAGCTTCAAG-3′), were described previously (4). We designed the primers 16S rDNA R-2F (5′-GAAGATTTCTTCTCGTTTCCGC-3′), 16S rDNA R-2R (5′-GTCTTGCTTCCCTCTGTAAC-3′), rompA-Fb (5′-GGTGCGAATATAGACCCTGA-3′), and rompA-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.

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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 *Alu* 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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Dr. Gaowa is an associate professor in Inner Mongolia Key Laboratory of Tick-Borne Zoonosis, Department of Medicine, College of Hetao, Bayan Nur, Inner Mongolia, China. Her primary research interests are molecular biology, ecology, and epidemiology of zoonotic parasites, especially tickborne pathogens.

**References**


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

<table>
<thead>
<tr>
<th>Patient type or tick species</th>
<th>No. tested</th>
<th>Rickettsia raoultii</th>
<th>R. aeschlimannii</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis-seronegative patients</td>
<td>261</td>
<td>6 (2.3)</td>
<td>0</td>
<td>6 (2.3)</td>
</tr>
<tr>
<td>Ticks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hyalomma asiaticum</em></td>
<td>766</td>
<td>118 (15.4)</td>
<td>0</td>
<td>118 (15.4)</td>
</tr>
<tr>
<td><em>Hyalomma marginatum</em></td>
<td>198</td>
<td>0</td>
<td>160 (80.8)</td>
<td>160 (80.8)</td>
</tr>
<tr>
<td><em>Dermacentor nuttalli</em></td>
<td>1,418</td>
<td>830 (58.5)</td>
<td>158 (11.1)</td>
<td>988 (68.7)†</td>
</tr>
<tr>
<td><em>Rhipicephalus turanicus</em></td>
<td>76</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total ticks</td>
<td>2,458</td>
<td>948 (38.6)</td>
<td>318 (12.9)</td>
<td>1,266 (51.5)</td>
</tr>
</tbody>
</table>

*SFGR, spotted fever group rickettsiae.†We did not detect dual infection with *R. raoultii* and *R. aeschlimannii* in *D. nuttalli* ticks in this study.*
Japanese Spotted Fever in Eastern China, 2013

This research letter describes the isolation of Rickettsia japonica from a febrile patient in Lu’an City, China, in 2013. Subsequently, an R. japonica seroprevalence of 54.8% (494/902) was found in the rural population of Anhui Province. R. japonica is tickborne, obligately intracellular, gram-negative bacteria with a worldwide distribution. This 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, 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. In this study, a 61-year-old man reported several tick bites 10 days before the onset of his illness, but 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. 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 (100 × 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 (130 × 10^9/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 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 and by electron microscopy. Electron microscopy showed the bacilli localized to the cytoplasm. We isolated Rickettsia japonica from the 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.

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