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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′-CGAACCTAGCCGTATTAGAATG-3′) and *gltA*-Rc (5′-CTTTAGAGGAGTACCTCAAG-3′), were described previously (4). We designed the primers 16S rDNA R-2F (5′-GAAGATTTCCTTCCGTTCTCGCC-3′), 16S rDNA R-2R (5′-GTCTTGGCTTCCCTGTAAAC-3′), ompA-Fb (5′-GGTGCGAATATAGACCCTGA-3′), and ompA-Ra (5′-TTAGTCCTAGAGGCTGACCA-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 *Rickettsia* sp. XY99 (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.
To assess patients’ risk of infection with SFGR by tick exposure, we collected 2,458 ticks morphologically identified as *Hyalomma asiaticum* (n = 766), *Hyalomma marginatum* (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. We detected rickettsial DNA in *R. raoultii* (GenBank accession no. DQ365803) or to that of *R. aeschlimannii* (GenBank accession no. HM050276) (Table; online Technical Appendix Figure 2). 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 the 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* (118/766, 15.4%) 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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**References**

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.

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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 gltA 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.

References
