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## Japanese Spotted Fever in Eastern China, 2013

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DOI: <https://doi.org/10.3201/eid2411.170264>

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.

<sup>1</sup>These 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 ( $10.34 \times 10^9$  cells/L), increased neutrophils (87.5%), and a platelet count within reference range ( $130 \times 10^9$ /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  $\mu$ mol/L, reference range 53–106  $\mu$ 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  $\leq 0.15$  ng/mL) and an interleukin 6 concentration of 52 pg/mL (reference range  $\leq 1.8$  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

but mainly had dimensions  $0.2 \mu\text{m} \times 0.5\text{--}1 \mu\text{m}$  (online Technical Appendix Figure 3).

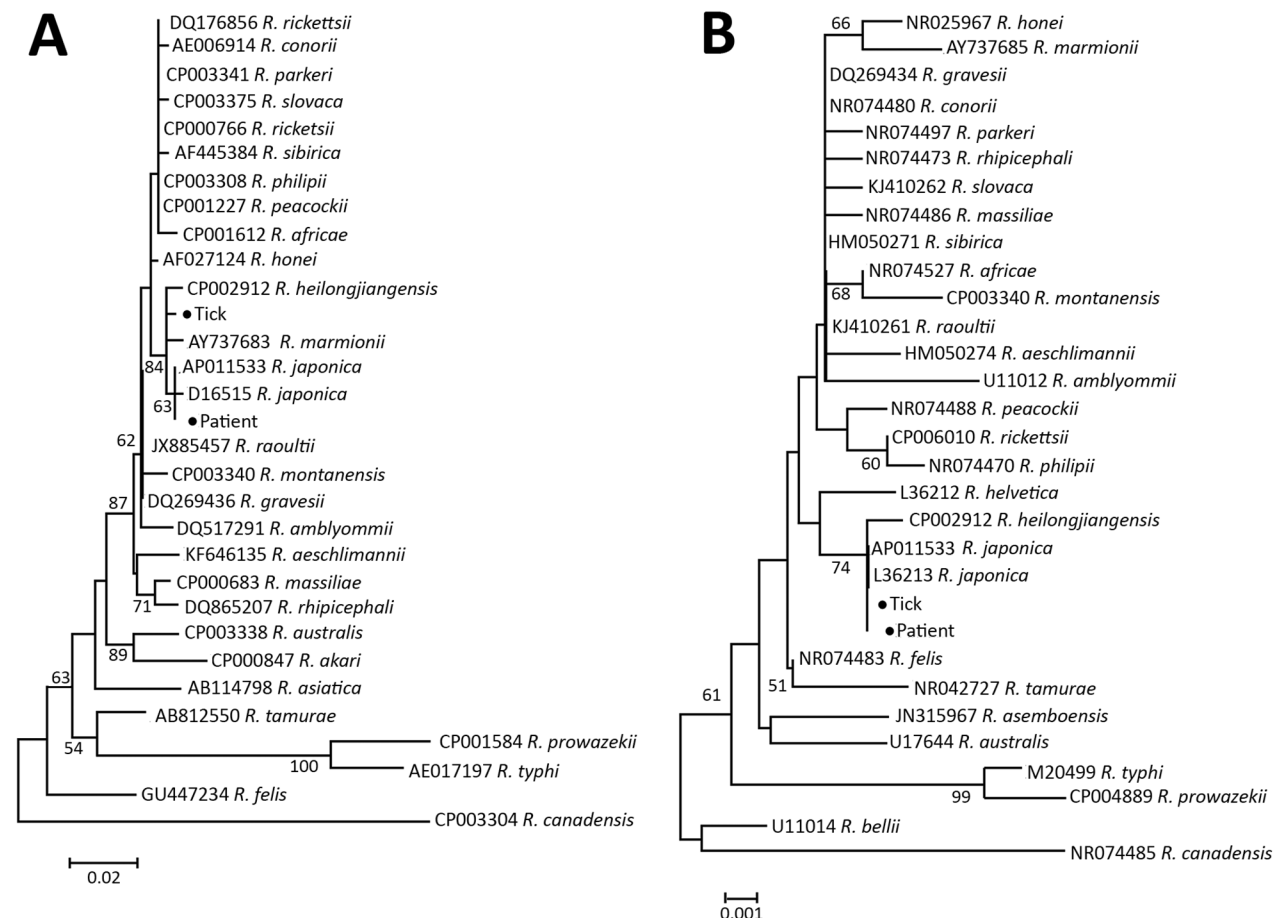
We amplified and sequenced the 17-kDa protein gene, 16S rRNA gene, *ompA*, *ompB*, and gene D of *R. japonica* (GenBank accession nos. KY364904, KY484160, KY484162, KY484163, and KY488633; online Technical Appendix Table). These gene sequences were 99.8%–100% homologous with the corresponding gene of an *R. japonica* isolate (GenBank accession no. AP017602.1).

Hard-body tick species *Haemaphysalis longicornis*, *H. flava*, and *Dermacentor taiwanensis* (5,6) have been reported as *R. japonica* transmission vectors. We acquired questing *H. longicornis* ticks in Shandong Province, China, in 2013 and found them positive for the *R. japonica* 17-kDa protein and 16S rRNA genes by PCR (online Technical Appendix). The percentage of *H. longicornis* ticks infected with *R. japonica* rickettsia in Shandong Province was 0.5% (5/975). The *H. longicornis* tick, which is prevalent in East China and feeds on domestic animals and small mammals, might be a major vector of *R. japonica* rickettsia in China

(7,8). Phylogenetic analysis of the 16S rRNA (Figure, panel A) and 17-kDa protein (Figure, panel B) genes indicated that the rickettsial isolates from the patient and *H. longicornis* tick were identical to *R. japonica* isolates and in the same clade with *R. heilongjiangensis*.

Examination by indirect immunofluorescence assay showed that the patient's acute (1:80 dilution) and convalescent (1:1,280 dilution) serum samples reacted to isolated antigen of *R. japonica* bacterium. During 2013, we collected serum samples from 902 healthy persons living in rural areas of Anhui Province (online Technical Appendix Figure 1) and tested them with the same assay. We found 54.8% (494/902) of serum samples positive for *R. japonica*-specific antibodies.

In summary, we detected *R. japonica* bacteria in a patient and an *H. longicornis* tick and demonstrated high *R. japonica* seroprevalence among the rural population of Anhui Province. In agreement with Lu et al.'s work in 2015 (9), our findings suggest that *R. japonica* might be more prevalent in China than previously thought. Physicians in



**Figure.** Phylogenetic analysis of *Rickettsia* isolate from patient with Japanese spotted fever in Anhui Province and isolate from *Haemaphysalis longicornis* tick in Shandong Province, China, 2013 (black dots), compared with reference isolates. Unrooted neighbor-joining trees of 16S rRNA gene (A) and 17-kDa protein gene (B) were constructed by using MEGA 5.2 (<https://www.megasoftware.net/>) and 1,000 bootstrap replications. Scale bars represent substitutions per nucleotide.

China need to become aware of *R. japonica* disease presentation, so they can administer the appropriate treatment to patients with suspected *R. japonica* infections.

This study was supported by the National Natural Science Foundation of China (81571963); Science Foundation of Anhui Province of China (1608085MH213); Natural Science Foundation Key Project of Anhui Province Education Department (KJ2015A020, KJ2016A331); and Scientific Research of Anhui Medical University (XJ201314, XJ201430, XJ201503).

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## **Burkholderia lata** Infections from Intrinsically Contaminated Chlorhexidine Mouthwash, Australia, 2016

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DOI: <https://doi.org/10.3201/eid2411.171929>

*Burkholderia lata* was isolated from 8 intensive care patients at 2 tertiary hospitals in Australia. Whole-genome sequencing demonstrated that clinical and environmental isolates originated from a batch of contaminated commercial chlorhexidine mouthwash. Genomic analysis identified efflux pump–encoding genes as potential facilitators of bacterial persistence within this biocide.

*Burkholderia contaminans* and *B. lata* together form group K of the *B. cepacia* complex (Bcc). These predominantly environmental species are a major cause of pharmaceutical contamination and have been linked to multiple instances of associated opportunistic infection (1). Although both species are capable of causing serious infections in humans (2,3), only *B. contaminans* has been associated with infection outbreaks (3,4). We report a healthcare-associated *B. lata* infection outbreak occurring in intensive care units (ICUs) in 2 tertiary hospitals in Australia.

During May–June 2016, bacterial contamination of chlorhexidine mouthwash (0.2% mg/mL) was associated with an interjurisdictional outbreak in New South Wales and South Australia. Bcc isolates were obtained from blood and tracheal aspirates from 6 ICU patients in hospital A (South Australia) (sample information and isolation protocols detailed in the online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/11/17-1929-Techapp1.pdf>). An investigation by the hospital’s infection and prevention control team noted discoloration of a commercial chlorhexidine mouthwash. Bcc isolates were cultured from

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## Technical Appendix

### Methods

#### Patient and Healthy Population

We obtained the patient's clinical data from Shucheng County People's Hospital (Anhui Province, China). We also collected 902 serum samples from healthy persons in rural areas of Anhui Province, China, during August–December 2013. All participants provided written informed consent for collection and testing of blood samples. The research protocol was approved by the human bioethics committee of Anhui Medical University.

#### Isolation of Pathogen

An anticoagulated acute blood sample was obtained from the patient on day 1 after hospitalization and was inoculated onto monolayers of Vero E6 cells and THP-1 cells in 12.5-cm<sup>2</sup> flasks. The THP-1 cells were cultured with RPMI (Roswell Park Memorial Institute) 1640 medium with 10% fetal bovine serum, and Vero E6 cells were cultured with minimum essential media with 5% fetal bovine serum. Cells were incubated at 35°C in an atmosphere of 5% carbon dioxide. A smear from each flask was stained with Diff-Quick (Thermo Fisher Scientific, Kalamazoo, MI, USA) every day and observed by using light microscopy to determine the presence of intracellular bacteria.

### **Serologic Testing**

Serum samples from the patient and healthy persons were tested for IgG specific to *Rickettsia* bacteria by indirect immunofluorescence assay. We seeded slides with Vero E6 cells and infected cells with purified *Rickettsia* bacteria isolated during this study. Serum samples were diluted 4 times in 2-fold increments (1:80–1:1,280). A person with a reciprocal titer of  $\geq 1:80$  was considered seropositive.

### **Transmission Electron Microscopy**

The isolated organism was cultivated in THP1 cells in a 25-cm<sup>2</sup> flask. When 90% of the cells were infected, the cells were harvested and incubated with a fixative solution containing 2.5% formaldehyde, 0.1% glutaraldehyde, 0.03% CaCl<sub>2</sub>, and 0.03% trinitrophenol in 0.05 M cacodylate buffer, pH 7.4. After fixation, the sample was stained with 1% osmium tetroxide in 0.05 M cacodylate buffer (pH 7.2) en bloc for 20 minutes with 2% aqueous uranyl acetate at 60°C, dehydrated in a graded series of ethanol, and embedded in Poly/Bed 812 (Polysciences Inc., Warrington, PA, USA). Ultra-thin sections were cut on a UC7 ultra microtome (Leica, Wetzlar, Germany), stained with lead citrate, and examined in a Philips 201 transmission electron microscope (Eindhoven, the Netherlands) at 60 kV.

### **Tick Samples**

Questing ticks were collected during June and July 2013 in Shandong Province, China, by flagging over vegetation. The ticks were frozen at –80°C until use. Tick species and developmental stages were identified morphologically, and the tick species was molecularly confirmed. A total of 975 ticks (540 nymphs and 435 adult ticks) were pooled into groups (containing either 20 nymphs or 5 adult ticks) and used for DNA extraction.

### **PCR Amplification and DNA Sequencing**

DNA was extracted from cell culture materials with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA samples were tested by PCR with primers designed to

amplify 5 rickettsial genes: 16S rRNA gene, 17-kDa protein gene, gene D, *ompA*, and *ompB* (Technical Appendix Table). Both strands of the PCR products were sequenced and compared with those in GenBank by using Blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### Phylogenetic Analysis

The DNA sequences of the 17-kDa protein gene and 16S rRNA gene of the *Rickettsia* species isolated in this study and DNA sequences of *Rickettsia* spp. from the GenBank database were aligned and compared by MEGA (<https://www.megasoftware.net/>). Phylogenetic trees were constructed by using the neighbor-joining method in MEGA 5 with 1,000 bootstrap replications.

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**Technical Appendix Table.** Primers for amplification and sequencing of genes of *Rickettsia* spp. isolated from patient with Japanese spotted fever, Anhui Province, China, 2013

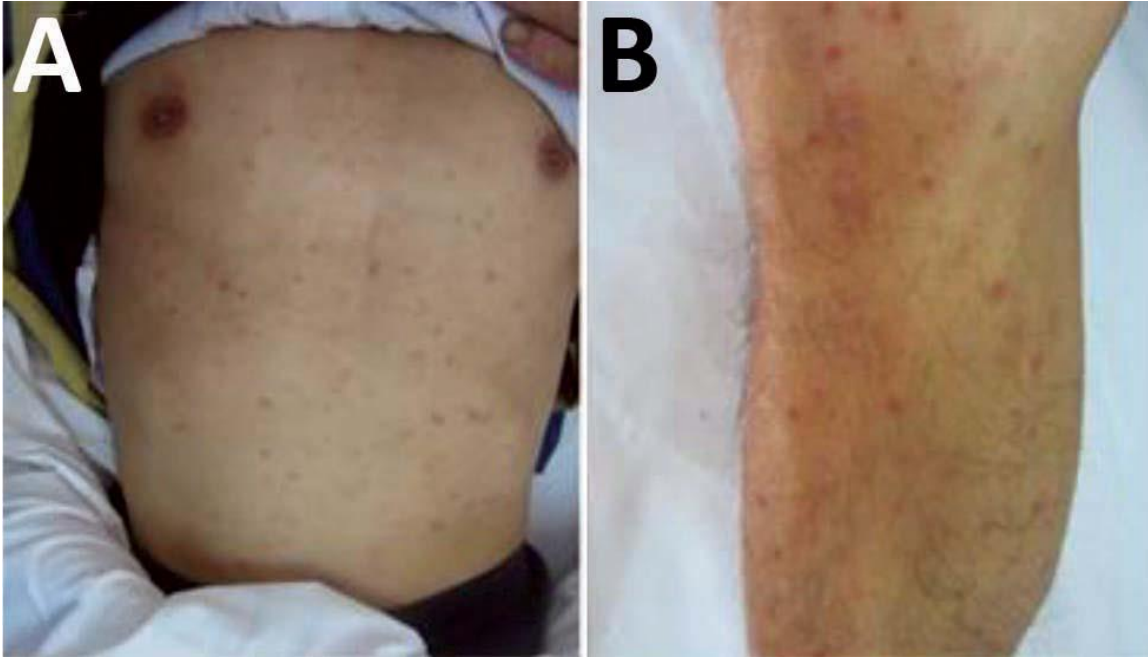
| Gene           | Primer name | Nucleotide sequence         | Position    | Reference |
|----------------|-------------|-----------------------------|-------------|-----------|
| <i>ompA</i>    | 190.70f     | 5'-ATGGCGAATATTTCTCCAAA-3'  | 70–90       | (1)       |
|                | 190.701r    | 5'GTTCCGTTAATGGCAGCATCT-3'  | 701–681     |           |
| <i>ompB</i>    | M59f        | 5'CCGCAGGGTTGGTAACTGC-3'    | M59–M41     | (2)       |
|                | 807r        | 5'CCTTTTAGATTACCGCCTAA-3'   | 807–788     |           |
| 16S rRNA       | fD1out-f    | 5'-AGAGTTTGATCCTGGCTCAG-3'  | 339–357     | (3)       |
|                | 357f        | 5'TACGGGAGGCAGCAG-3'        | 334–348     |           |
|                | 800r        | 5'CTACCAGGGTATCTAAT-3'      | 828–812     |           |
|                | 1050r       | 5'CACGAGCTGACGACA-3'        | 1,109–1,095 |           |
|                | rP2-out-r   | 5'-ACGGCTACCTTGTTACGACTT-3' | 1,406–1,392 |           |
| 17-kDa protein | 17kD-f      | 5'GCTCTTGCAACTTCTATGTT-3'   | 31–50       | (4)       |
|                | 17KD-r      | 5'CATTGTTTCGTCAGGTTGGCG-3'  | 464–445     |           |
| D              | D1f         | 5'ATGAGTAAAGACGGTAACCT-3'   | 1–20        | (5)       |
|                | D928r       | 5'-AAGCTATTGCGTCATCTCCG-3'  | 928–907     |           |



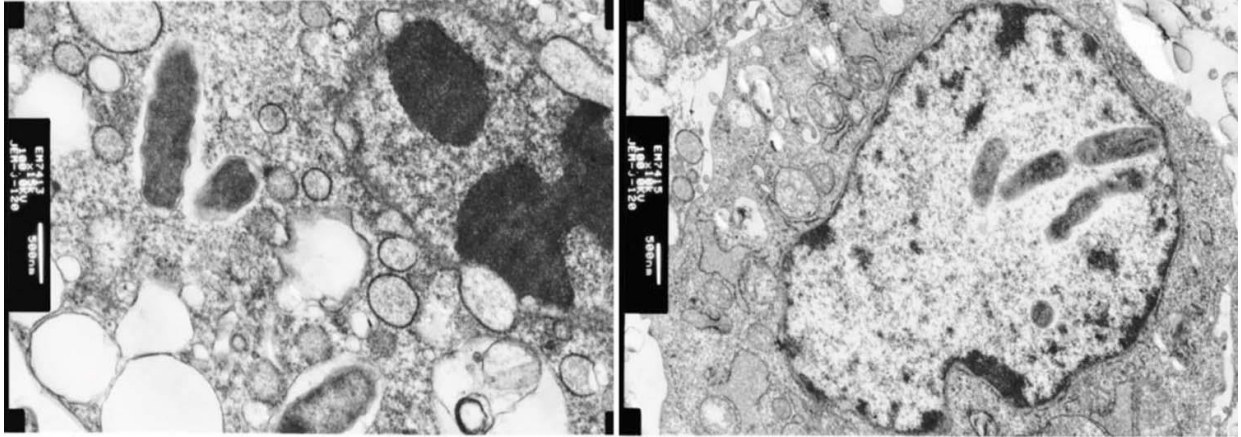


**Technical Appendix Figure 1.** Location of patient with Japanese spotted fever in 2013 and collection sites for serum samples from healthy persons, Anhui Province, China. The location of Anhui Province in China is indicated on the left. The location of the patient's hometown is marked with a clover. The serum samples were tested by an indirect immunofluorescence assay that included the bacteria isolated from the patient with Japanese spotted fever as the antigen. The percentage of healthy persons with antibodies specific to this pathogen and numbers of positive and total samples are indicated.





**Technical Appendix Figure 2.** Rash on 61-year-old-man with Japanese spotted fever, Anhui Province, China, August 2013. Papular rash on trunk (A) and leg (B) of patient.



**Technical Appendix Figure 3.** Electron micrograph of etiologic agent isolated from blood sample of patient with Japanese spotted fever, Anhui Province, China, August 2013. Patient blood sample was collected on day 1 after hospital admission, and pathogen was cultured in THP-1 cells. The bacterium was observed in the cytoplasm and nucleus by electron microscopy. Bar represents 0.5  $\mu\text{m}$ .