Clinical Forms of Japanese Spotted Fever from Case-Series Study, Zigui County, Hubei Province, China, 2021

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We report a case-series study of 5 patients with Japanese spotted fever from the Three Gorges Area in China, including 1 fatal case. Seroprevalence of *Rickettsia japonica* was ≈12% among the local population. Our report highlights the emerging potential threat to human health of Japanese spotted fever in the area.

Japanese spotted fever (JSF), caused by the bacterium *Rickettsia japonica*, was first described in 1984 in Japan (*1*). It has been recognized in multiple countries in Asia, including Japan, South Korea, the Philippines, Thailand, and China (*1*–*5*), suggesting that it is endemic in Asia. In China, *R. japonica* has been detected in ticks from the central, southeast, and northeast regions (*6*–*8*). Since JSF cases were first found in Anhui Province in 2013 (*5*), a total of 39 have been reported in the Dabie Mountains on the borders of Henan, Anhui, and Hubei Provinces and in the Tianmu Mountains in Zhejiang Province on the eastern coast (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/1/22-0639-App1.pdf) (*9*–*11*).

The Study

Our study was approved by the ethics committees of the National Institute for Communicable Disease Control and Prevention (ICDC). During April–October 2021, five JSF cases were found in Zigui County, Hubei Province, in the Three Gorges area of China (Appendix Figure 1), including case 1, in which the patient died because of multiorgan failure. Among the 5 case-patients (3 female, 2 male), disease onset occurred during April–October (2 cases in spring, 3 in autumn); median age of case-patients was 53 years (range 47–70 years) (Table 1). All had histories of activity in fields, but none was aware of any tick bites. All initially manifested signs and symptoms of sudden fever (≥38.5°C), headache, chills, fatigue, chest tightness, shortness of breath, and dyspnea. All had rash and eschar (Appendix Figure 2), except for case-patient 1, who had no eschar. Case-patients 1, 3, and 5 experienced vomiting and case-patient 3 had abdominal pain. Medical laboratory tests (Table 2) showed that case-patients 1, 3, and 5 had thrombocytopenia and lymphopenia, case-patients 1 and 5 had neutrophilia, and case-patients 1 and 4 had mild anemia. All 5 patients had remarkably elevated levels of C-reactive protein and D-dimer, and except for case-patient 5, all had elevated liver-associated enzymes. Proteinuria was detected in case-patients 1, 2, 3, and 5. Case-patients 1, 3, and 5 had simultaneous edema and effusion and case-patient 5 showed multiple effusions (pelvic, pleural, pericardial, and ascites).

Most laboratory tests were flagged as abnormal for case-patient 1, who died. She received an initial antimicrobial treatment (Appendix) at the village clinic without a causative agent being identified and already had severe sepsis (sequential organ failure assessment score = 12) by the time she was admitted to the hospital on day 8 after symptom onset. Disseminated intravascular coagulation was diagnosed, and she died of multiple organ failure on day 9 despite

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Table 1. Epidemiologic and clinical characteristics of patients with *Rickettsia japonica* infection, Zigui County, Hubei Province, China

Characteristics	Case 1*	Case 2	Case 3	Case 4	Case 5
Sex	Female	Male	Male	Female	Female
Age, y	58	47	68	57	70
Month of admission	Apr	Jun	Sep	Sep	Oct
Occupation	Farmer	Village teacher	Farmer	Farmer	Farmer
Possible causative exposures	Picking tea, tea garden	Collecting bamboo, bamboo groves	Herding sheep, woodland	Farm work, fields	Farm work and chopping wood, fields and woodland
Previous illness	Healthy	Healthy	Hypertension	Healthy	Hypertension
Day of admission†	8	4	6	15	5
Signs and symptoms					
Highest temperature, °C	39.0	39.0	39.0	38.5	39.5
Fever type	Continued	Continued	Continued	Undetermined‡	Undetermined§
Headache	Present	Present	Present	Present	Present
Malaise	Present	Present	Present	Present	Present
Myalgia	Present	Present	Present	Present	Present
Chills	Present	Present	Present	Present	Present
Eschar	Absent	Present	Present	Present	Present
Rash (day)	Present (11)	Present (3)	Present (10)	Present (13)	Present (10)
Hypotension (day)	Present (9)	Absent	Absent	Absent	Absent
Dyspnea (day)	Present (9)	Present (6)	Present (10)	Present (15)	Present (5)
Vomiting (day)	Present (9)	Absent	Present (10)	Absent	Present (7)
Edema (day)	Face (9)	Absent	Lower extremities (10)	Absent	Lower extremities (10)
Clouding of consciousness	Present	Present	Present	Present	Present
Proteinuria	+++	+–	+	–	++
Anuria (day)	Present (9)	Absent	Absent	Absent	Absent
Abdominal pain	Absent	Absent	Present (11)	Absent	Absent
Pelvic effusion	Absent	Absent	Absent	Absent	Present (5)
Pleural effusion (day)	Present (9)	Absent	Present (10)	Absent	Present (5)
Pericardial effusion	Absent	Absent	Present (10)	Absent	Present (5)
Ascites effusion	Absent	Absent	Absent	Absent	Present (5)

*Fatal case.
†Day on which sign or symptom manifested in patient, when applicable. The day when the patients first experienced headache and fatigue was defined as Day 0.
‡Difficulty determining fever type because of drug intervention.
§Urine protein concentration: –, <0.1 g/L; +–, 0.1–0.2 g/L; +, 0.2–1.0 g/L; ++, 1.0–2.0 g/L; +++, 2.0–4.0 g/L.

continuous intensive therapy. The other 4 patients recovered without sequelae after receiving doxycycline or minocycline treatment (Appendix).

Using a PCR assay, we detected 6 rickettsial genes (*groEL*, *glt*A, *omp*A, *omp*B, *sca*4, and 17kDa gene) in a blood specimen from case-patient 1. In addition, we

Table 2. Laboratory findings of the patients with *Rickettsia japonica* infection, Zigui County, Hubei Province, China*

Laboratory findings*	Reference value	Case 1	Case 2	Case 3	Case 4	Case 5
Leukocyte, $\times 10^9$ cells/L	4.0–10.0	9.09	3.50	7.78	8.45	9.15
Hemoglobin, g/L	110–150 (F), 120–160 (M)	109	130	127	105	115
Platelets, $\times 10^9$/L	100–300	45	99	72	110	82
Lymphocytes, $\times 10^9$ cells/L	0.8–3.5	0.52	0.72	0.91	2.29	0.64
Neutrophils, $\times 10^9$ cells/L	1.8–6.3	8.11	2.55	5.80	5.80	7.90
CRP, mg/L	0.8–8.0	199.53	72.8	77.00	72.00	111.72
PCT, μg/L	<0.5	5.64	0.38	2.14	0.4	0.54
D-dimer, μg/mL	0–0.5	4.200	2.081	2.160	1.090	1.080
TBil, mg/L	3–13	38.0	12	12	9.3	11
DBil, mg/l	0–2.5	24.0	2.0	3.4	2.7	2.0
TBA, μmol/L	<10	43.1	3.9	8.6	4.8	4.9
TP, g/L	60–80	48.7	62.9	52.3	58.2	65
Albumin, g/L	35–55	23.8	36.2	30.6	35.6	35.9
A/G	1.5–2.5	1.0	1.4	1.4	1.6	1.2
ALT, U/L	0–35	21	69	177	167	16
AST, U/L	0–40	114	88	167	111	29
BUN, mmol/L	2.9–7.5	7.81	3.81	10.80	2.38	7.54
Creatinine, mg/L	40–100 (F), 50–110 (M),	93.2	84.9	74.1	58.2	83.9
Uric acid, μmol/L	150–357 (F), 200–416 (M)	378	301	259	182	210
LDH, U/L	100–300	587	412	403	433	368
Creatine kinase, U/L	18–198	257	186	51	48	125
α-HBDH, U/L	90–220	401	294	254	317	323
Sodium, mmol/L	135–145	137.7	141.9	132.9	131.4	136.5

*α-HBDH, α-hydroxybutyrate dehydrogenase; A/G, albumin/globulin ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; DBil, direct bilirubin; LDH, lactate dehydrogenase; TBA, total bile acids; TBil, total bilirubin; TP, total protein.

amplified *groEL*, *glt*A, and 17kDa genes from case-patients 2, 3, and 5 but only *glt*A and 17kDa from case-patient 4. For all 5 patients, the genomic sequences of each amplified target gene were 100% identical to each other; phylogenetic analysis revealed that the causative agent was most closely related to *R. japonica* (Figure). We submitted the obtained sequences to GenBank (accession nos. OM966424–8 for *glt*A,

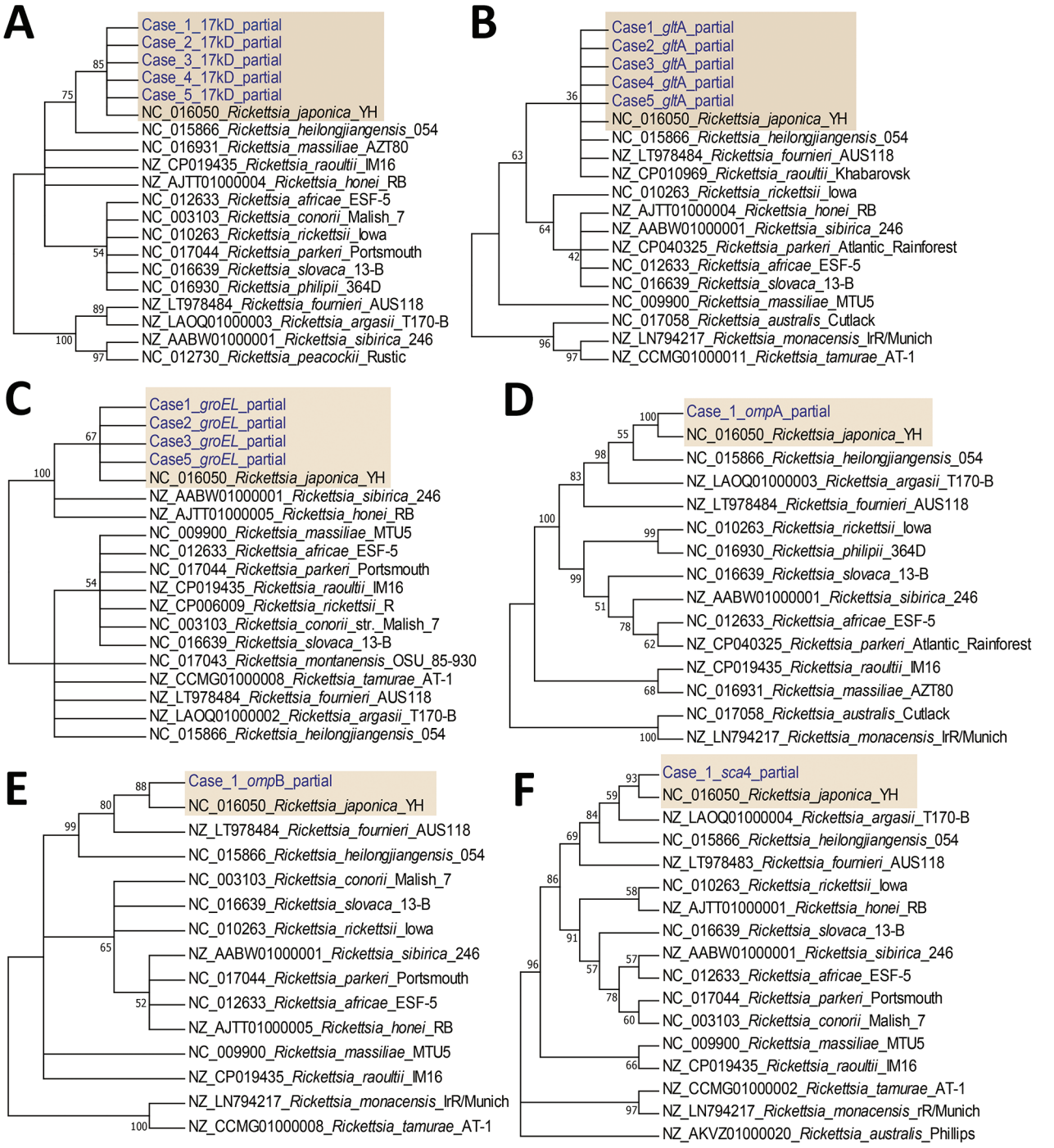

Figure. Bootstrap consensus phylogenetic tree constructed based on partial sequences of 17kDa (A), *glt*A (B), *groEL* (C), *omp*A (D), *omp*B (E), and *sca*4 (F) amplified from blood specimens from 5 spotted fever patients in Japan (yellow shading). We aligned sequences using MUSCLE within MEGA6 software (http://www.megasoftware.net). We analyzed phylogenetic relationships using the neighbor-joining method with 1,000 bootstrap replicates; boot values are shown next to the branches. Genbank accession numbers for the *Rickettsia* strains retrieved are indicated.

OM966422 for *omp*A, OM966423 for *omp*B, OM966412–6 for 17kDa, OM966417 for *sca*4, and OM966418–21 for *groEL*). We obtained 1 stable rickettsial isolate, designated *R. japonica* strain YC21, from the blood of case-patient 1 using Vero cell cultures (Appendix Figure 3) and obtained the whole genomic sequence from the isolate (GenBank BioProject PRJNA812951). Phylogenetic analysis based on core genes suggested that *R. japonica* strain YC21 was most closely related to *R. japonica* strain LA16/2015 (Appendix Figure 4); 30 virulence-associated genes of *R. japonica* strain YC21 (Appendix Table 2), predicted using the virulence factor database (http://www.mgc.ac.cn/VFs), were completely homologous to those of strain LA16/2015.

We used an immunofluorescence assay using *R. japonica* strain YC21 as coating antigen (a cutoff of 1:64 was determined by testing negative and positive samples) and *R. rickettsii* (Focus Diagnostics, http://focusdiagnostics.in) to test serum-specific antibodies from the 5 JSF patients and 100 healthy subjects recruited locally. Case-patients 2 and 3 were confirmed to have JSF on the basis of a ≥4-fold increase in *R. rickettsii*–specific and *R. japonica*–specific IgG titers between acute and convalescent phase serum (Appendix Table 3). At baseline, 12/100 (12%) of the healthy local donors tested positive (range, 1:128–1:1,024; geometric mean, 512) for *R. japonica*–specific IgG.

We measured cytokine and chemokine levels in the serum samples collected from the JSF patients (during acute phase) and 6 healthy donors (Appendix Table 4). The levels of interferon (IFN) γ, interleukin (IL) 6, IL-10, IL-1α, macrophage inflammatory protein 1β, IL-8, IFN gamma-induced protein 10, and monocyte chemoattractant protein 1 in the 4 surviving JSF patients were significantly higher than in the healthy donors ($p < 0.01$), consistent with previous reports, except for the exclusion of tumor necrosis factor α (*12*–*14*). In the case-patient who died, serum levels of IL-6, IL-10, and IFN-γ were 10-fold higher than those in the surviving case-patients and the levels of IL-4, INF-α, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein 1, macrophage inflammatory protein 1β, and IP-10 were 2-fold higher, suggesting that *R. japonica* infection might cause an unregulated hyperinflammatory state, potentially leading to cytokine release syndrome (*14*).

Conclusion

We identified 5 cases of JSF, including 1 in which the patient died, in Zigui County in the Three Gorges Area of China, where JSF has not previously been identified. Furthermore, our study revealed a high prevalence (12%) of *R. japonica* among residents, suggesting a new endemic area for JSF in China and indicating that JSF might be more widespread than previously thought. We should be alert to the potential risk for JSF, especially in areas where *R. japonica* is detected in vectors (Appendix Figure 1). The JSF cases were confirmed by PCR detection and serologic tests. A strain of *R. japonica* isolated from the blood of the patient who died was revealed to be most closely related to strains LA16/2015 and LA4/2015 detected in Zhejiang Province, suggesting that a virulent strain of *R. japonica* might have spread widely across China.

Delayed treatment is one of the worst prognostic factors for patients with JSF, and as a neglected infectious disease, it might not be considered during differential diagnosis. In our study, the patient who died manifested a faint rash, but without eschar, which resulted in delayed diagnosis and provision of correct antimicrobial treatment when she first visited the rural clinic. Profiling the patient's serum cytokine and chemokine levels indicated notably elevated IL-6, IL-10, and IFN-γ, characteristic of potential cytokine release syndrome. The primary findings on patient cytokines levels benefit understanding of immune response to *R. japonica* infection.

Our findings highlight the threat of JSF to public health in China. Healthcare workers, especially in rural areas where residents are at increased risk for tick exposure, should be aware of this potentially deadly infectious disease. Long-term surveillance and investigation of local hosts and vectors of *R. japonica* are necessary to improve the prevention and treatment of JSF.

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Appendix

Materials and Methods

Molecular Diagnosis and Amplicon Sequencing

For molecular diagnosis, the DNA extracted from each blood sample was first screened for the presence of zoonotic pathogens (*Rickettsia* spp., *Orientia tsutsugamushi*, *Anaplasma* spp., *Ehrlichia* spp., *Borrelia* spp., *Bartonella henselae*, *Francisella* spp., *Coxiella burnetii*, *Chlamydia psittaci*) by real-time PCR (*1–6*). After other pathogens were ruled out, the DNA samples was subjected to PCR analysis using primer pairs designed to target conserved rickettsial species genes (*glt*A, *omp*A, *omp*B, *sca*4, and 17kD) (Appendix Table 1) (*7–9*). The resulting PCR products were sequenced by Sanger sequencing and sequences were aligned against the GenBank database using the "BLAST" tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic trees were generated by the maximum likelihood tree method implemented in MEGA 6 software (http://www.megasoftware.net) with 1000 bootstrap replicates. A one-step RT-nested PCR method was also conducted to further test for severe fever with thrombocytopenia syndrome virus (SFTSV) specific RNA (*10*).

Rickettsial Isolation from the Blood Samples

A 12-well plate was seeded with Vero cells (2.5×10^5/well) suspended in minimal essential medium (DMEM) (Gibco, USA) supplemented with 2% fetal bovine serum (Gibco,

USA) and 2 mM glutamine (Sigma, USA) two days before infection. The blood clot homogenate was inoculated into each well (50μL/well), and then the plate was centrifuged in bucket rotors at 700×g for 30 minutes. The plate was incubated at 32ºC and 5% CO_2 for 3-5 days. When the culture medium was renewed every 4 days, the cells in each well were scratched manually with a streaking loop. The obtained cells were smeared on a slide and stained by Gimenez staining (Solarbio, China).

Isolation of DNA

Genomic DNA of isolated Rickettsia strain was prepared as previously described (*11*). Mainly, rickettsiae was first released from infected Vero cells by a tissue grinder. Host cell debris was removed by centrifugation at 270 rcf for 5 minutes, and the supernatant was filtered through a 2.0 μm filter unit (Jinteng, Tianjin, China). Rickettsiae were recovered from the filtrate by centrifugation (17,000 rcf, 15 min 4°C) and resuspended in D-PBS with calcium and magnesium (Beyotime, Shanghai, China). The free host DNA were digested by treating with DNase I (15 μg/ml; from bovine pancreas Type II-S, Sigma-Aldrich) for 30 min at room temperature. After DNase I treatment rickettsiae were centrifuged again (17,000 rcf, 15 min 4°C) and genomic DNA was extracted using QIAamp Tissue kit (QIAGEN, USA) and quantified using a Qubit fluorometer (Life Technologies, Paisley, UK). The DNA sample was aliquoted and stocked at –80ºC until use. The whole genome of *R. japonica* str. YC21 was sequenced using MinION Nanopore (Oxford Nanopore Technologies, Oxford, UK) and Illumina Hiseq (Illumina, San Diego, CA, USA) methods.

MinION Sequencing

The library was prepared for MinION Nanopore sequencing using a Genomic DNA ligation kit (SQK-LSK109; Oxford Nanopore Technologies) according to the manufacture's protocols. DNA libraries were loaded onto FLO-MIN106 flow cells (R9.4.1), and sequenced using the MinION Mk1C for 72 h.

Illumina Sequencing

The genomic DNA was randomly sheared into short fragments. The obtained fragments were end repaired, A-tailed, and further ligated with Illumina adapter. The short-insert (≈500 bp) libraries were constructed as described in Illumina library preparation kit. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. The constructed library was sequenced on an Illumina iSeq 100 platform (San Diego, CA, USA). The raw reads were subsequently trimmed for quality using Trimmomatic (v.0.35) (*12*) with the parameters "ILLUMINACLIP: TruSeq3-PE.fa:2:30:10 LEADING: 3 TRAILING: 3 SLIDINGWINDOW: 4:15 MINLEN: 36". The clean data obtained from this process were used for subsequent analysis.

Genome Assembly

Base calling of the fast5 files was performed using GUPPY (version 1.4.3-1; Oxford Nanopore Technologies). Reads were then BLAST searched against the NCBI nucleotide (nt) database (*13*). All long reads related to the genus *Rickettsia* were mapped to the reference *Rickettsia japonica* str. YH genome sequence (GenBank accession number NC_016050) using Minialign 0.5.3 (*14*) and coverage plots were visualized using Geneious v11.1 (*15*). To improve the accuracy of our assembly, the whole-genome Illumina short reads were mapped to the Oxford Nanopore long reads using BWA-MEM and errors were corrected (*16*). Rawdata was submitted to National Genomics Data Center (https://ngdc.cncb.ac.cn/gsa/browse/CRA006321).

Phylogenetic Analysis of *Rickettsia japonica*

Genome sequences of 34 *Rickettsia japonica* strains were downloaded from the NCBI. Snippy (v4.6.0) (*17*) was used to finds SNPs between the YC21 and the reference genomes to generate a core SNP alignment. The recombinant regions were filtered with Gubbins (v2.4.1) (*18*). Maximum likelihood phylogenetic trees were estimated using FastTree (v.2.1.10) (*19*).

Medication of Patients

Case 1 first visited the village infirmary, complaining of a high fever, where she received an intravenous infusion of cefuroxime (1500 mg bid.), levofloxacin (400 mg qd), and ribavirin (500 mg qd). For the next two days, the dose of ribavirin was increased to 600–700 mg qd. She was then referred to the emergency ward of our hospital and treated with an intravenous infusion of ceftazidime (2000 mg q12h) and gastric administration of minocycline (100 mg qd). The patient died of multiple organ failure 2 days after admission.

Three patients (Cases 2, 4 and 5) received doxycycline (first dose 200 mg po, followed by 100 mg q12h). Case 3 received minocycline (first dose 200 mg po, followed by 100 mg tid).

Determination of Cutoff Values for IFA for JSF Diagnosis

R. japonica str. YC21 was co-cultivated in Vero cell culture to prepare antigen using T75 vented flasks and DMEM supplemented with 2% FBS. *R. japonica* str. YC21 co-cultured in Vero cells was grown in vented tissue culture flasks at 32ºC in an incubator with 5% CO_2. Briefly, when gross cytopathic effect was evident, we harvested infected cells by 0.25% trypsin digestion. The cell concentration was adjusted to $1.2–1.8\times10^5$/mL with DMEM medium containing 2% FBS. Twenty microliters of the cell suspension was added to each well of 12-well slides, and the slides were then air-dried, fixed in acetone and stored at –20°C until they were used.

Sera from 30 anonymous healthy donors from Beijing (no JSF case reported), China and 15 JSF patients (collected by our team and diagnosed via PCR) were used to determine the cutoff values for IFA. The cut-off value was determined following the previous reports (*20*).

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Appendix Table 1. Primers for PCR and sequencing

Primer	Target gene	Sequence (5'-3')	References‡
RpCS780p*	*glt*A	GACCATGAGCAGAATGCTTCT	*(7)*
RpCS1258n*		ATTGCAAAAAGTACAGTGAAC	
RpCS877p†		GGGGGCCTGCTCACGGCGG	
RpCS1258n†		ATTGCAAAAAGTACAGTGAAC	
Rr190k.71p*	*omp*A	TGGCGAATATTTCTCCAAAA	*(7)*
Rr190k.720n*		TGCATTTGTATTACCTATTGT	
Rr190k.71p†		TGGCGAATATTTCTCCAAAA	
Rr190k.602n†		AGTGCAGCATTCGCTCCCCCT	
Rgroel-OF*	*groEL*	AAGAAGGMGTGATAAC	*(8)*
Rgroel-OR*		ACTTCMGTAGCACC	
Rgroel-IF†		GATAGAAGAAAAGCAATGATG	
Rgroel-IR†		CAGCTATTTGAGATTTAATTTG	
R005F	*omp*B	GTAACCGGAAGTAATCGTTTCGTAAA	*(9)*
R005R		CTTTATAACCAGCTAAACCACCTT	
R010F	17kDa	GCTCTTGCAACTTCTATGTTACA	*(9)*
R010R		CATTGTTCGTCAGGTTGGCGgCATG	
R011F	*sca*4	ATGAGTAAAGACGGTAACCT	*(9)*
R011R		AAGCTATTGCGTCATCTCCG	

*Nested PCR primers, 1st round

†Nested PCR primers, 2nd round

‡Appendix Reference

Appendix Table 2. Virulence-associated genes predicted from *Ricettsia japonica* YC21 genome

VFclass	Virulence factors	Related genes
Actin-based motility	RickA	*rick*A
	Sca2	*sca*2
Adherence and invasion	Adr1	*adr*1
	Adr2	*adr*2
	Sca1	*sca*1
	Sca4	*sca*4
	rOmpA/Sca0	*omp*A
	rOmpB/Sca5	*omp*B
Enzyme	Hemolysin C	*tly*C
	Phospholipase A2	*pat*1
		*pat*2
	Phospholipase D	*pld*
Secretion system	Rvh T4SS effector	*ral*F
	Rvh T4SS	*rvh*B10
		*rvh*B11
		*rvh*B1
		*rvh*B2
		*rvh*B3
		*rvh*B4a
		*rvh*B4b
		*rvh*B6a
		*rvh*B6b
		*rvh*B6c
		*rvh*B6d
		*rvh*B6e
		*rvh*B8a
		*rvh*B8b
		*rvh*B9a
		*rvh*B9b
		*rvh*D4

Appendix Table 3. Serum immunoglobulin antibody titers of the Japanese spotted fever cases*

	Time of sample collection†		IgM (AP)			IgG (AP/CP)		
Cases	AP	CP	*R. rickettsii*	*R. typhi*	*R. japonica*‡	*R. rickettsii*	*R. typhi*	*R. japonica*‡
Case 1	10	NA	ND	ND	ND	ND/NA	ND/NA	ND/NA
Case 2	7	30	64	ND	64	64/512	ND/128	128/1024
Case 3	10	30	256	64	256	1024/4096	512/1024	1024/>4096
Case 4	14	NA	128	ND	256	1024/NA	128/NA	2048/NA
Case 5	10	NA	128	ND	256	2048/NA	512/NA	2048/NA

*AP, acute phase; CP, convalescent phase; IFA, immunofluorescence assay; NA, not available; ND, none detected

†Days after first symptom onset

‡Coating with isolated *R. japonica* str. YC21

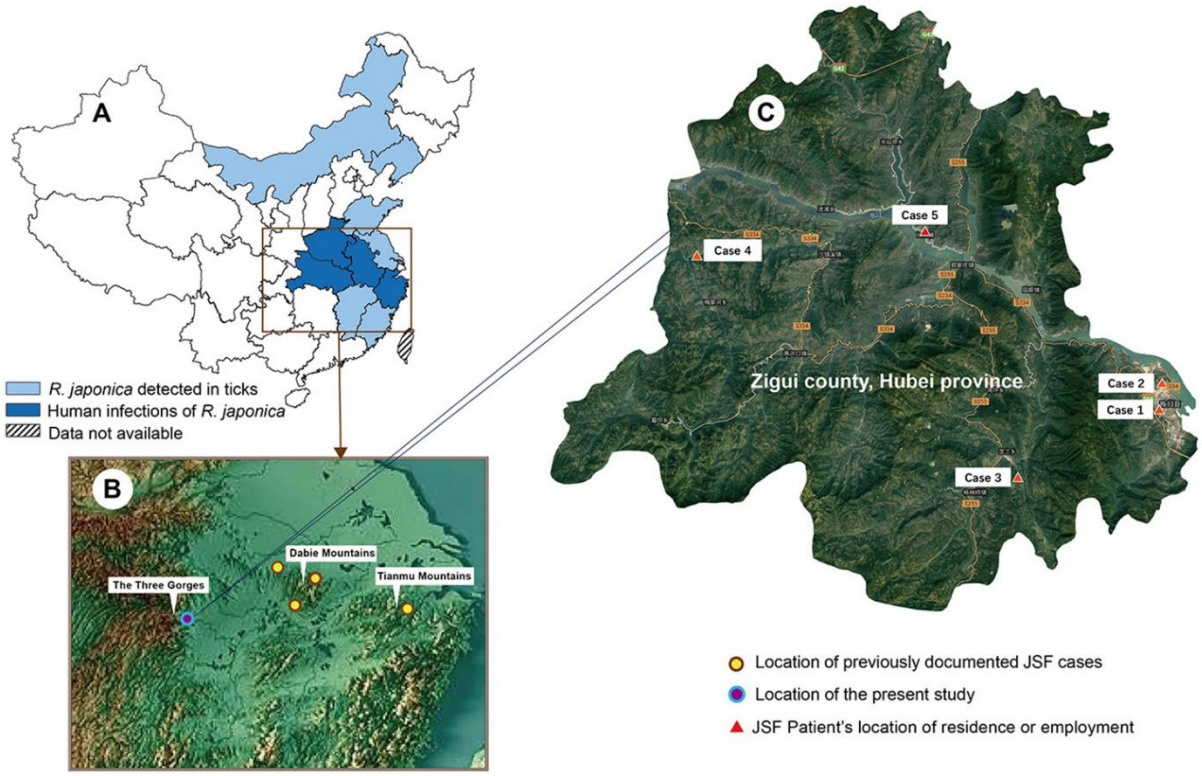
Appendix Table 4. Serum cytokine and chemokine concentrations (ng/μL) *

		Case-patients†		Control participants			
Type	Identity	Mean	SD	Mean	SD	p-value‡	Case-patient 1
Cytokines	IFN-γ	28.95	0.40	20.20	5.10	<0.01	417.67
	TNF-α	47.55	7.28	69.31	23.50		85.86
	IL-8(CXCL-8)	674.35	291.80	19.27	12.42	<0.01	585.05
	IL-12p70	12.45	2.01	18.49	3.61		23.80
	IL-1β	1.92	0.15	2.07	0.49		3.93
	IL-4	13.51	2.77	10.66	2.64		34.02
	IL-6	31.93	5.46	5.10	1.24	<0.01	647.17
	IL-17A(CTLA-8)	11.20	2.55	17.68	4.81		16.23
	IL-10	22.70	2.24	0.89	0.37	<0.01	406.85
	IL-13	3.87	1.10	6.00	1.50		3.52
	GM-CSF	5.16	0.32	6.49	0.29	<0.01	10.61
	IFN-α	2.27	0.45	2.41	0.70		5.81
	IL-1α	12.02	4.83	2.81	3.64	<0.01	13.55
Chemokines	MIP-1α(CCL3)	101.46	70.32	31.12	20.11		104.25
	MIP-1β	504.99	281.83	110.70	36.49	<0.01	935.94
	IP-10(CXCL10)	431.79	28.04	27.56	15.62	<0.01	961.01
	MCP-1(CCL2)	53.29	30.36	112.60	16.27	<0.01	226.69

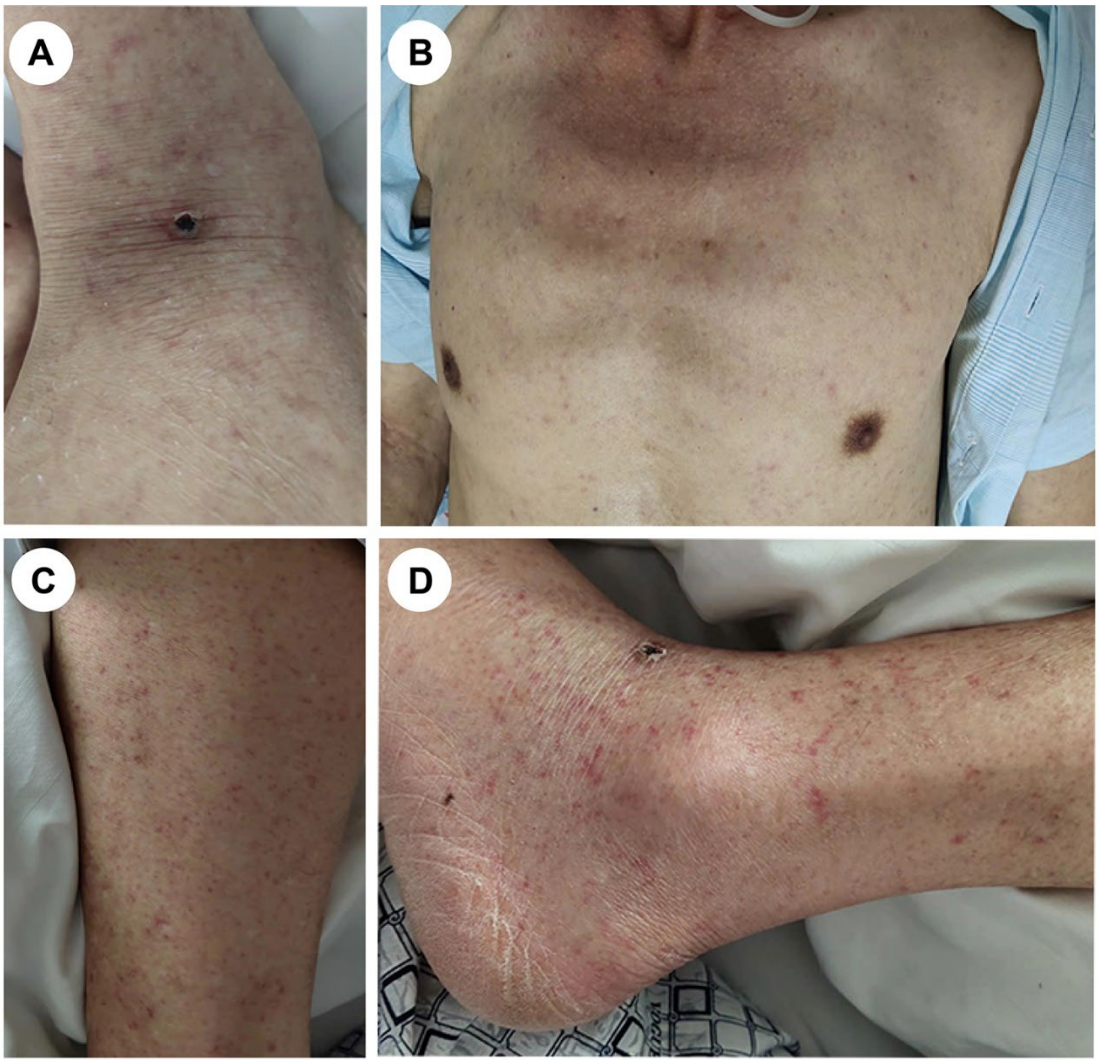
*SD, standard deviation

†Four surviving case-patients (2, 3, 4, and 5).

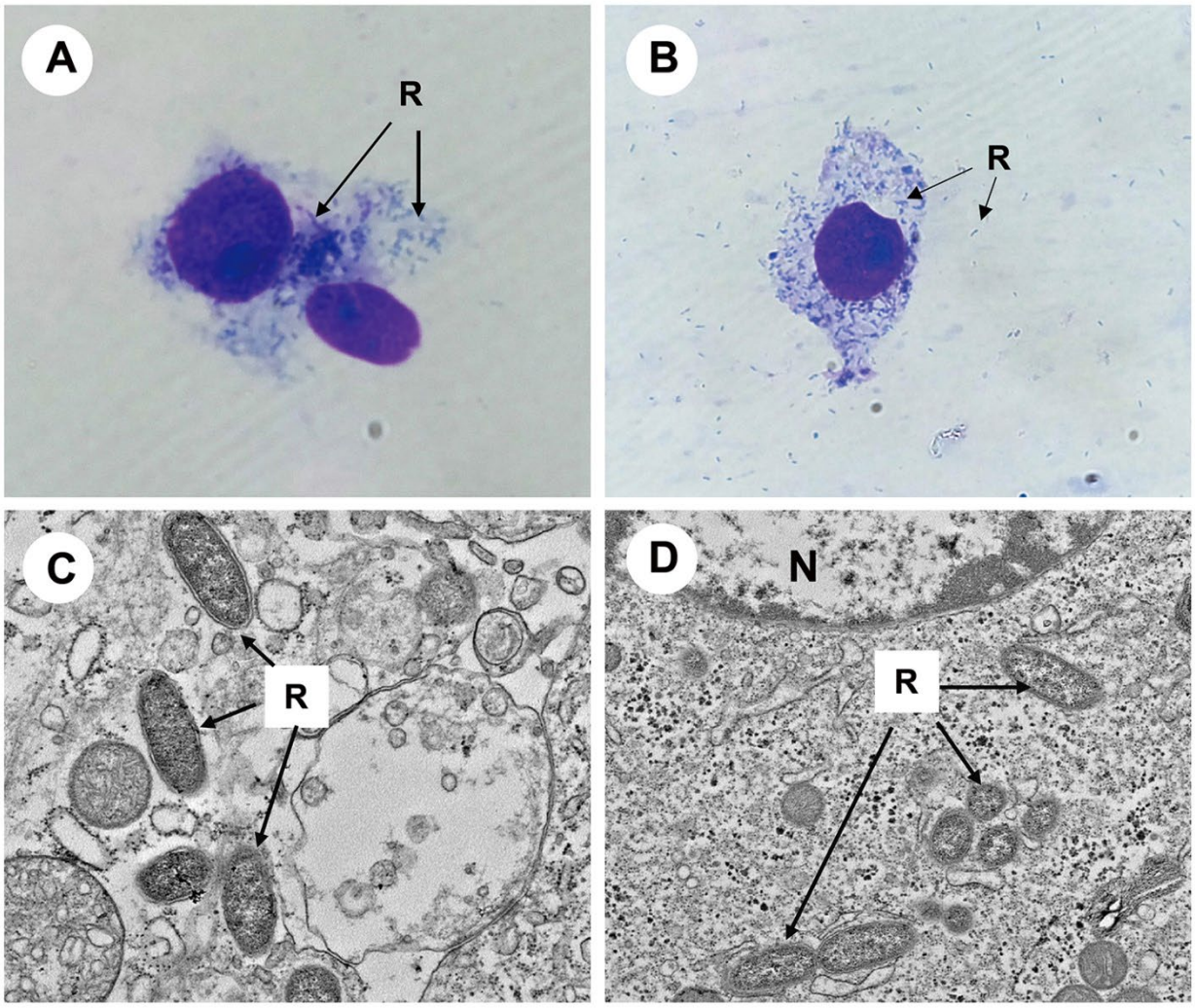
‡Four surviving case-patients (2, 3, 4, and 5) compared with controls analyzed using a paired Student's t-test in Microsoft Excel.



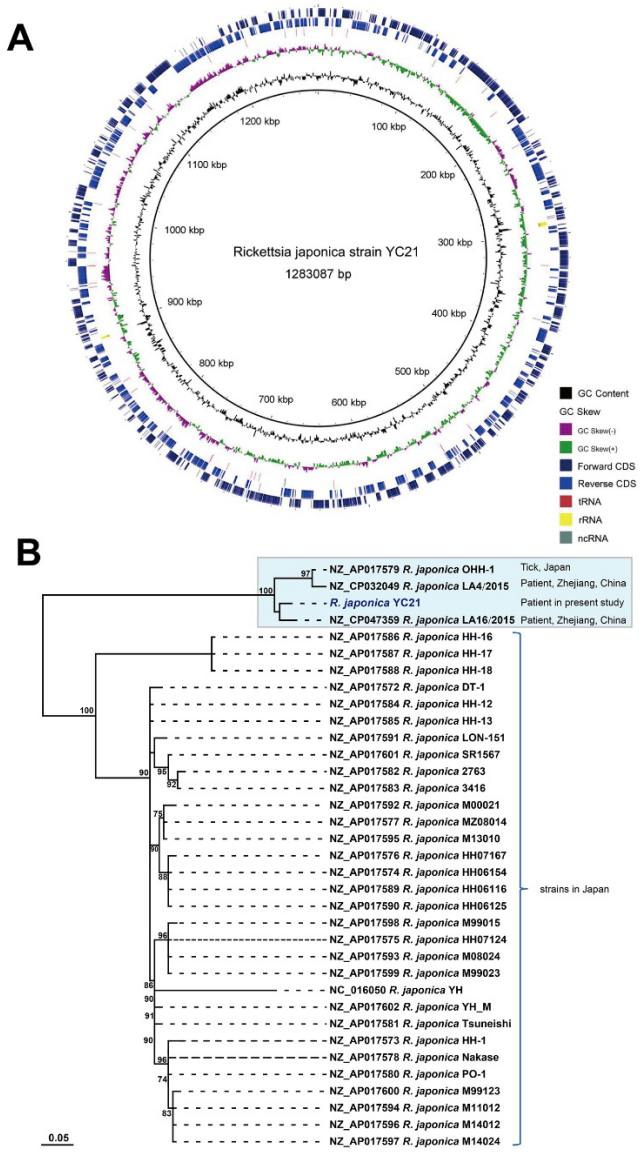
Appendix Figure 1. *Rickettsia japonica* in mainland China. A) Distribution of *Rickettsia japonica.* Provinces where *R. japonica* has not been detected are white, if detected in ticks, light blue, if from human-related samples, deep blue. Out of 34 provinces and special administrative regions, 10 (29.4%) are *R. japonica*–positive, and 4 provinces had human infections. B) Japanese spotted fever cases found in China. The reported Japanese spotted fever cases in the present work were in the Three Gorges Area, a mountainous area with large water systems. Previously documented Japanese spotted fever cases in China were distributed in the Dabie and Tianmu Mountains. C) Map of Zigui County. Most cases were from the river valley area.



Appendix Figure 2. A) Eschar on the left upper ankle; pink-red spotted rashes on B) the chest, C) calves, and D) left foot of a patient with Japanese spotted fever (case-patient 3)



Appendix Figure 3. Photomicrographs of cells infected with *Rickettsia japonica* strain YC21 isolated from blood of case-patient 1. A) and B) giemsa-stained Vero cells under a light microscope; C) and D) Vero cells infected with *R. japonica* strain YC21 under a scanning electron microscope.



Appendix Figure 4. Genome of *Rickettsia japonica* strain YC21 is most closely related to that of *Rickettsia japonica* strain LA16/2015. A) Genome of *R. japonica* strain YC21. Whole genomic sequence of *R. japonica* strain YC21 was 1,283,531 bases with 32.4% GC content. Structural annotation of the genome revealed a total of 1,439 coding sequences and 40 RNAs consisting of 33 transfer RNAs, 3 ribosomal RNAs (23S, 16S and 5S rRNA), 3 noncoding RNAs, and 1 transfer-messenger RNA; B) Phylogenetic tree for core genes of *R. japonica* strains. An unrooted phylogenetic tree generated on the basis of the genome shows the evolutionary relationships across *R. japonica* strains. Scale bar represents nucleotide distance.