Human Infection with Novel Spotted Fever Group *Rickettsia* Genotype, China, 2015

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Only 4 species of spotted fever group rickettsiae have been detected in humans in China. However, phylogenetic analysis of samples from 5 ill patients in China indicated infection with a novel spotted fever group *Rickettsia*, designated *Rickettsia* sp. XY99. Clinical signs resembled those of severe fever with thrombocytopenia syndrome.

Spotted fever group (SFG) rickettsiae are globally distributed and mostly transmitted by ticks (*1*). Recently, emerging and reemerging SFG rickettsiae, such as *Rickettsia slovaca* (*2*), *R. aeschlimannii* (*3*), *R. massiliae* (*4*), *Candidatus* Rickettsia tarasevichiae (*5*,*6*), and *R. sibirica* subspecies *sibirica* BJ-90 (*7*), previously considered nonpathogenic, were found to infect humans. In addition, *R. parkeri* was confirmed to be pathogenic 65 years after its detection in ticks in 1939 (*8*).

In China, SFG rickettsioses are not listed as reportable diseases, and only 4 species of SFG rickettsiae (*R. heilongjiangensis*, *R. sibirica* subspecies *sibirica* BJ-90, *Candidatus* Rickettsia tarasevichiae, and *R. raoultii*) have been detected in human blood samples (*9*). In contrast, besides these pathogenic species, at least 4 other species of SFG rickettsiae (*R. sibirica* subspecies *mongolotimonae*, *R. monacensis*, *R. slovaca*, *Candidatus* Rickettsia hebeiii) have been detected in ticks, urging a wider search for cases in humans. We report infection of 5 patients with a novel SFG rickettsia in eastern central China.

The Study

From March through November 2015, at the People's Liberation Army 154 Hospital in Xinyang City, Henan Province, China, patients who were acutely symptomatic with fever and had a history of tick bites or animal contact within the past month were screened for SFG rickettsiae infection. At admission, EDTA-anticoagulated samples of peripheral blood were collected. DNA was extracted by using a QIAamp DNA Blood Mini Kit (QIAGEN, Germantown, MD, USA). Nested PCRs selective for outer membrane protein A (*omp*A) and citrate synthase (*glt*A) genes were concurrently performed to detect SFG rickettsial DNA (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/12/16-0962-Techapp1.pdf)). Positive amplicons were purified and then sequenced in both directions. Acute-phase (≤7 days after illness onset) and convalescent-phase (≥14 days after illness onset) serum samples were tested by indirect immunofluorescence assay (IFA) for IgG against *R. rickettsii* by using a commercially available IFA kit (Focus Diagnostics Inc., Cypress, CA, USA).

Positive amplification of *omp*A and *glt*A genes was found for 5 patients, and the obtained sequences for each of the 2 genes from all 5 patients were identical. Nucleotide sequence (350-bp) of *omp*A gene (GenBank accession no. KU853020) from each of the 5 patients showed 10-bp differences from that of *R. massiliae* strain AZT80 (GenBank accession no. CP003319) and 12-bp differences from that of *R. rhipicephali* strain HJ#5 (GenBank accession no. CP013133). Nucleotide sequences (1150-bp) of *glt*A gene (GenBank accession no. KU853022) from each of the 5 patients differed from that of *R. massiliae* strain AZT80 by 4 bp and from that of *R. rhipicephali* strain HJ#5 by 5 bp (online Technical Appendix Table 2). According to phylogenetic analysis, the novel SFG rickettsiae genotype, here designated as *Rickettsia* sp. XY99, seems to represent a distinct lineage and could constitute a new species (Figure 1). For all 5 patients, seroconversion or a 4-fold increase of IgG against *R. rickettsii* was found between the acute- and convalescent-phase samples, and the patients were determined to have acute infection with SFG rickettsiae (online Technical Appendix Table 3). Subsequent testing of the 5 patients for infection with severe fever with thrombocytopenia syndrome virus, *Anaplasma phagocytophilum*, "*A. capra*," and *Babesia microti* by molecular (real-time PCR or nested PCR) and serologic tests (ELISA or IFA) produced no positive results.

All 5 patients were farmers who resided in the villages of Xinyang City. Patient median age was 65 (range 62–80) years, and 3 were male (Table). Two patients had a history of tick exposure, and the other 3 had had contact with livestock. For all 5 patients, illness onset occurred June 20–July 10, 2015. The median time from illness onset to

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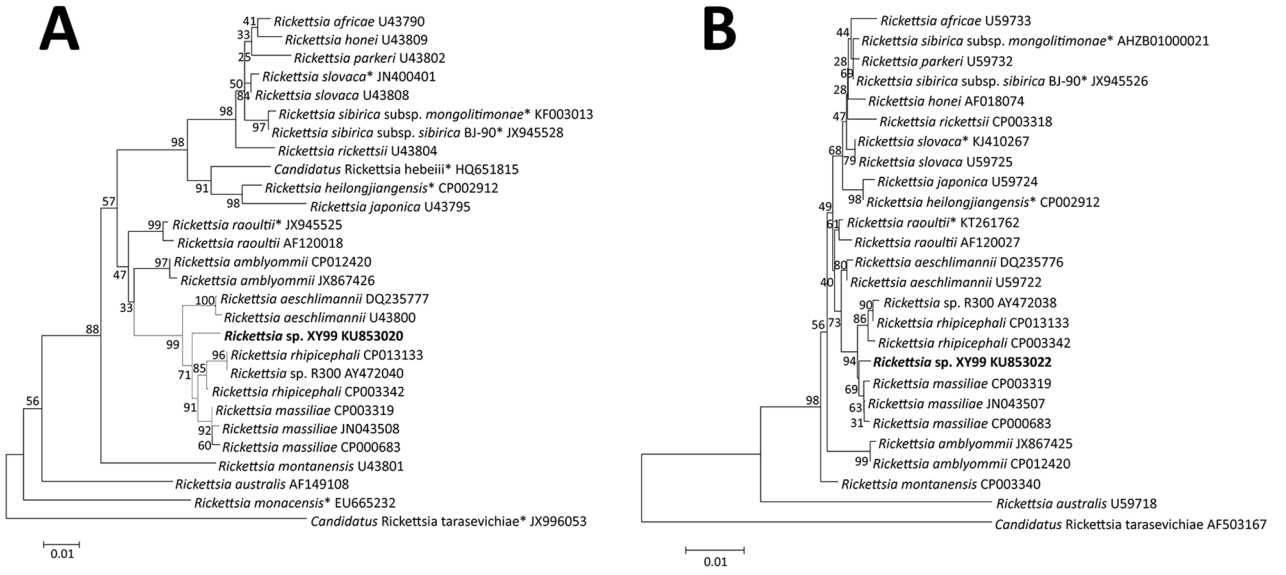

Figure 1. Phylogenetic analyses based on nucleotide sequences of the outer member protein A (307-bp) (A) and citrate synthase (1,150-bp) (B) genes of *Rickettsia*. Boldface indicates the newly discovered *Rickettsia* genotype (*Rickettsia* sp. XY99). Asterisks after taxon names indicate that the sequence of *Rickettsia* species was found in China. Neighbor-joining trees were conducted by using the maximum composite likelihood method by means of MEGA version 5.0 (http://www.megasoftware.net). Bootstrap analysis of 1,000 replicates was applied to assess the reliability of the reconstructed phylogenies. Scale bars indicate estimated evolutionary distance.

admission was 4 (range 3–6) days, and the median duration of hospitalization was 10 (range 8–12) days. All patients experienced fever (highest 38.4°C– 40.0°C), asthenia, anorexia, and nausea; 4 had cough, 3 vomiting, 2 myalgia, 1 headache, and 1 dizziness. Of note, all 5 patients had lymphadenopathy, but none had rash or eschar. At admission, all 5 patients had leukopenia, thrombocytopenia, and elevated hepatic aminotransferase levels; 4 had elevated

Table. Epidemiologic and clinical characteristics of 5 patients with *Rickettsia* sp. XY99 infection, China, 2015*

Characteristic	Patient no.				
	1	2	3	4	5
Age, y	65	64	66	80	62
Sex	M	F	F	M	M
History of tick bite	No	No	No	Yes	Yes
Time between tick bite and illness onset, d	NA	NA	NA	14	6
Time from onset to admission, d	3	6	5	4	4
Duration of hospitalization, d	12	8	9	12	10
Fever	Yes	Yes	Yes	Yes	Yes
Highest temperature, °C	40.0	39.5	38.7	38.4	39.1
Headache	Yes	No	No	No	No
Dizziness	No	Yes	No	No	No
Asthenia	Yes	Yes	Yes	Yes	Yes
Myalgia	Yes	Yes	No	No	No
Rash	No	No	No	No	No
Eschar	No	No	No	No	No
Lymphadenopathy	Yes	Yes	Yes	Yes	Yes
Anorexia	Yes	Yes	Yes	Yes	Yes
Nausea	Yes	Yes	Yes	Yes	Yes
Vomit	Yes	Yes	Yes	No	No
Cough	Yes	Yes	Yes	No	Yes
Pneumonia	Yes	No	Yes	No	Yes
Hydrothorax	Yes	No	Yes	No	No
Confusion	Yes	No	No	No	No
Meningeal irritation sign	Yes	No	No	No	No
Ecchymosis	Yes	No	No	No	No
Hemoptysis	No	No	No	No	Yes
Hematuria	Yes	Yes	No	No	No

*NA, not applicable.

lactate dehydrogenase levels, and 2 had elevated creatine kinase levels (Figure 2). Treatment included therapy with cefminox and cefoperazone; no doxycycline was used.

Complications included pneumonia (3 patients), hemorrhagic signs (3), hydrothorax (2), and neurologic syndromes (1). For 1 patient, severe complications progressively emerged 6 days after disease onset and included pneumonia and hydrothorax (online Technical Appendix Figure), confusion, meningeal irritation sign, ecchymosis, and hematuria. Laboratory indicators were substantially more out of range 7 days after disease onset, indicative of severe multiorgan dysfunction (Figure 2). Treatment was ineffective, and the patient died 15 days after disease onset. The other 4 patients were discharged after 8–12 days' hospitalization; at that time, all clinical signs and symptoms had resolved, but for certain patients, laboratory values remained out of reference range, suggesting slow recovery of organ dysfunction (Figure 2).

Conclusions

Our detection of *Rickettsia* sp. XY99 DNA in blood samples collected during the acute period of illness (days 3–6 after onset) from 5 patients in the same region of China suggests that this organism was the etiologic agent of the infection. Seroconversion or a 4-fold increase in titers of IgG against *R. rickettsii* provided supportive evidence of SFG *Rickettsia* infection. Phylogenetic analysis indicated that *Rickettsia* sp. XY99 was a novel genotype of SFG rickettsiae.

In contrast to humans with *R. massiliae* infection and many other SFG rickettsioses reported previously (*4*,*10*), none of the 5 patients infected with *Rickettsia* sp. XY99 had rash or eschar, and only 1 had headache. In recent years, the concept of the classic triad of fever, rash, and headache suggesting infection with SFG rickettsiae has been increasingly challenged. Several emerging SFG rickettsiae species, such as *R. slovaca* (*2*), *R. raoultii* (*11*), *R. africae* (*12*), and *R. helvetica* (*13*), can infect humans, but such infections lack these traditional features, which were also lacking in the cases reported here. Therefore, absence of rash and tick-bite history should not exclude suspicion of SFG rickettsiae infection.

Similar to *R. slovaca* and *R. raoultii* infections, which can be associated with tickborne lymphadenopathy and *Dermacentor*-borne necrosis-erythema-lymphadenopathy (*14*), lymphadenopathy was also observed in all 5 patients with *Rickettsia* sp. XY99 infection. Thus, lymphadenopathy might be a typical sign useful for clinical diagnosis of *Rickettsia* sp. XY99 infection. All 5 patients had gastrointestinal syndromes, indicating potential tissue lesions or vascular injury of the gastrointestinal tract. The hydrothorax

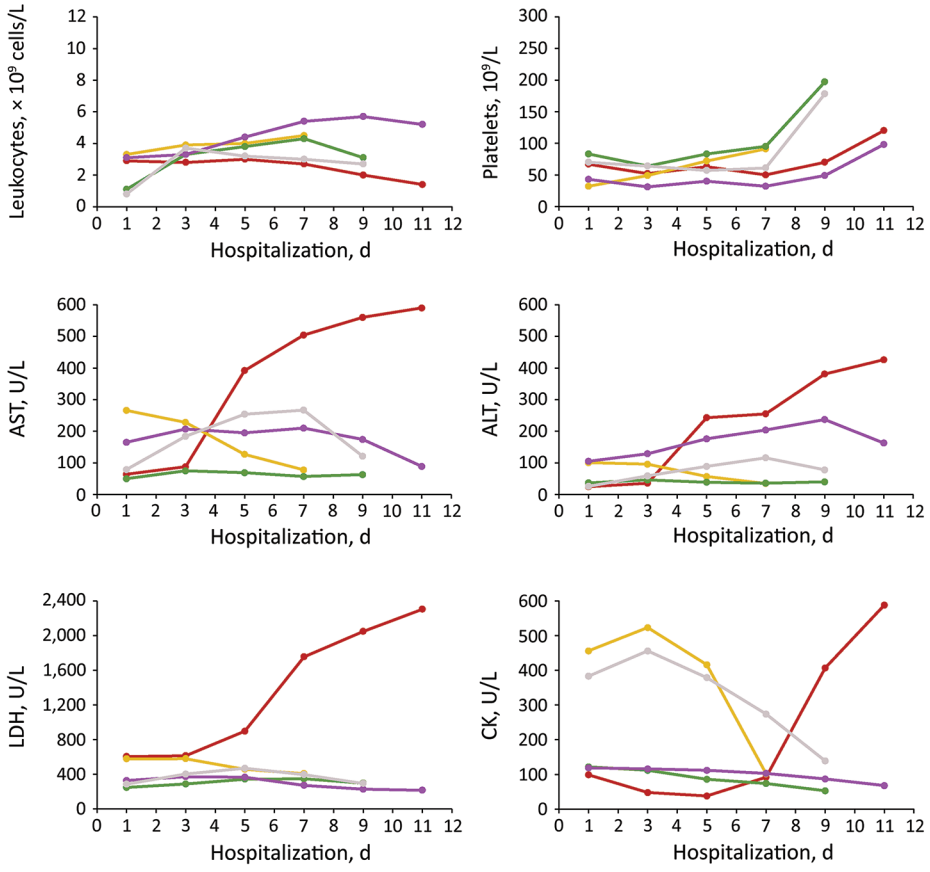

Figure 2. Dynamic changes of 6 laboratory parameters (with 2-day intervals) during hospitalization of 5 patients with *Rickettsia* sp. XY99 infection, China, 2015. Red, patient 1; yellow, patient 2; green, patient 3; purple, patient 4; gray, patient 5. ALT, alanine aminotransferase, reference range 0–40 U/L; AST, aspartate aminotransferase, reference range 0–40 U/L; CK, creatine kinase, reference range 25–200 U/L; LDH, lactate dehydrogenase, reference range 109–245 U/L; platelets, reference range 100–300 × 10^9/L; leukocytes, reference range 4.0–10.5 × 10^9 cells/L.

and multiple hemorrhagic signs in 4 patients is possibly suggestive of vascular invasion or damage caused by this novel *Rickettsia* species.

Confirmation of the novel *Rickettsia* genotype was achieved only by sequencing the *omp*A and *glt*A genes. Although differences based on 2 gene segments support its designation as a novel species, isolation efforts and characterization of all 5 genes (*rrs*, *glt*A, *omp*A, *omp*B, and *gene*D) are warranted, according to the guidelines for classification of a new *Rickettsia* species (*15*).

Physicians in this area of China should be aware of human infections with *Rickettsia* sp. XY99. It should be included in differential diagnoses with severe fever with thrombocytopenia syndrome, which causes similar clinical illness and is also endemic to the same area in eastern central China.

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Technical Appendix

Materials

Molecular detection of rickettsial infection

DNA was extracted from blood specimens collected at admission with the use of the QIAamp Blood Mini Kit (Qiagen) according to manufacturer's instructions. Nested PCR assays targeting the *ompA* and *gltA* genes were concurrently performed to detect the presence of SFG rickettsial DNA. Nucleotide sequences of the primers were shown in Technical Appendix Table 1.

Results

Serologic test results for 5 patients with *Rickettsia* sp. XY99 infection were shown in Technical Appendix Table 2.

Patient 1 developed pneumonia and hydrothorax as shown in the Technical Appendix Figure.

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Technical Appendix Table 1. Nucleotide sequences of the primers used in the study

Organism	Target gene	Primer	Sequence (5'-3')	Reference
Spotted fever group *Rickettsia*	*omp*A*	Rr190.70p	ATGGCGAATATTTCTCCAAAA	(*1*)
		Rr190.602n	AGTGCAGCATTCGCTCCCCCT	
		190.70-38s1	AAAACCGCTTTATTCACC	(*2*)
		190.602-384r1	GGCAACAAGTTACCTCCT	
	*glt*A†	CS1d	ATGACTAATGGCAATAATAA	(*3*)
		CSEndr	CTTATACTCTCTATGTACA	
		RpCS877p	GGGGACCTGCTCACGGCGG	
		RpCS1258n	ATTGCAAAAAGTACAGTGAACA	
Anaplasma phagocytophilum	*glt*A	W1	TGTTTTGGAGTGTGGAGAC	(*4*)
		W1	GGTGAACCAATCTCAGCAA	
		N1	ATATAGAAAATCTGATCGG	
		N2	CTCTAAGTTTGCGTCAGC	
"A. capra"	*glt*A	Outer-f	GCGATTTTAGAGTGYGGAGATTG	(*5*)
		Outer-r	TACAATACCGGAGTAAAAGTCAA	
		Inner-f	GGGTTCMTGTCYACTGCTGCGTG	
		Inner-r	TTGGATCGTARTTCTTGTAGACC	
Babesia microti	18S rRNA	Bab1	CTTAGTATAAGCTTTTATACAGC	(*6*)
		Bab4	ATAGGTCAGAAACTTGAATGATACA	
		Bab2	GTTATAGTTTATTTGATGTTCGTTT	
		Bab3	AAGCCATGCGATTCGCTAAT	
severe fever with thrombocytopenia	S-segment	Forward	TTCACAGCAGCATGGAGAGG	(*7*)
syndrome virus		Reverse	GATGCCTTCACCAAGACTATCAATG	
		Probe	FAM-AACTTCTGTCTTGCTGGCTCCGC-TAMRA	

*Nucleotide positions of the four primers are 1-21, 513-533, 52-69, and 381-398, referring to the *omp*A sequence of *Rickettsia heilongjiangensis* (GenBank accession number AF179362).

†Nucleotide positions of the four primers are 1-20, 1272-1290, 797-815, and 1157-1178, referring to the *omp*A sequence of *R. helvetica* (GenBank accession number KU310588).

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Technical Appendix Table 2. Sequence similarity of *omp*A and *glt*A genes between *Rickettsia* sp. XY99 and other *Rickettsia* strains

				*omp*A		*glt*A	
Species	Strain	Country	Host	GenBank accession number	Similarity	GenBank accession number	Similarity
R. massiliae	AZT80	USA	Human	CP003319	340/350	CP003319	1146/1150
	MTU5	Cyprus	Human	CP000683	339/350	CP003319	1146/1150
	56m	Italy	Human	KJ663747	340/350	-	-
	I20	Israel	Tick	KJ187077	339/350	-	-
	GL041	Guinea	Tick	JN043508	339/350	-	-
R. rhipicephali	3-7-6	-	-	CP003342	340/350	CP003342	1145/1150
	HJ#5	Brazil	Tick	CP013133	338/350	CP013133	1145/1150
	Do290	USA	Tick	EU109176	339/350	-	-
Rickettsia sp. R300	R300	Brazil	Tick	AY472040	338/350	AY472038	1144/1150
R. aeschlimannii	MC16	Morocco	Tick	U43800	335/350	U59722	1142/1150
	Stavropol	Russia	Tick	DQ235777	336/350	DQ235776	1141/1150
R. amblyommii	Ac37	Brazil	Tick	CP012420	327/350	CP012420	1134/1150

Technical Appendix Table 3. Serologic test results for 5 patients with *Rickettsia* sp. XY99 infection*

			Days after onset		IFA*	
Patient No.	Age	Sex	AP	CP	AP	CP
Patient 1	65	M	4	15	<64	256
Patient 2	64	F	7	14	64	256
Patient 3	66	F	6	14	<64	128
Patient 4	80	M	5	16	<64	128
Patient 5	62	M	5	14	<64	64

*Performed by detection of IgG against *R. rickettsia*. AP, acute phase; CP, convalescent phase; IFA, indirect immunofluorescence assay.

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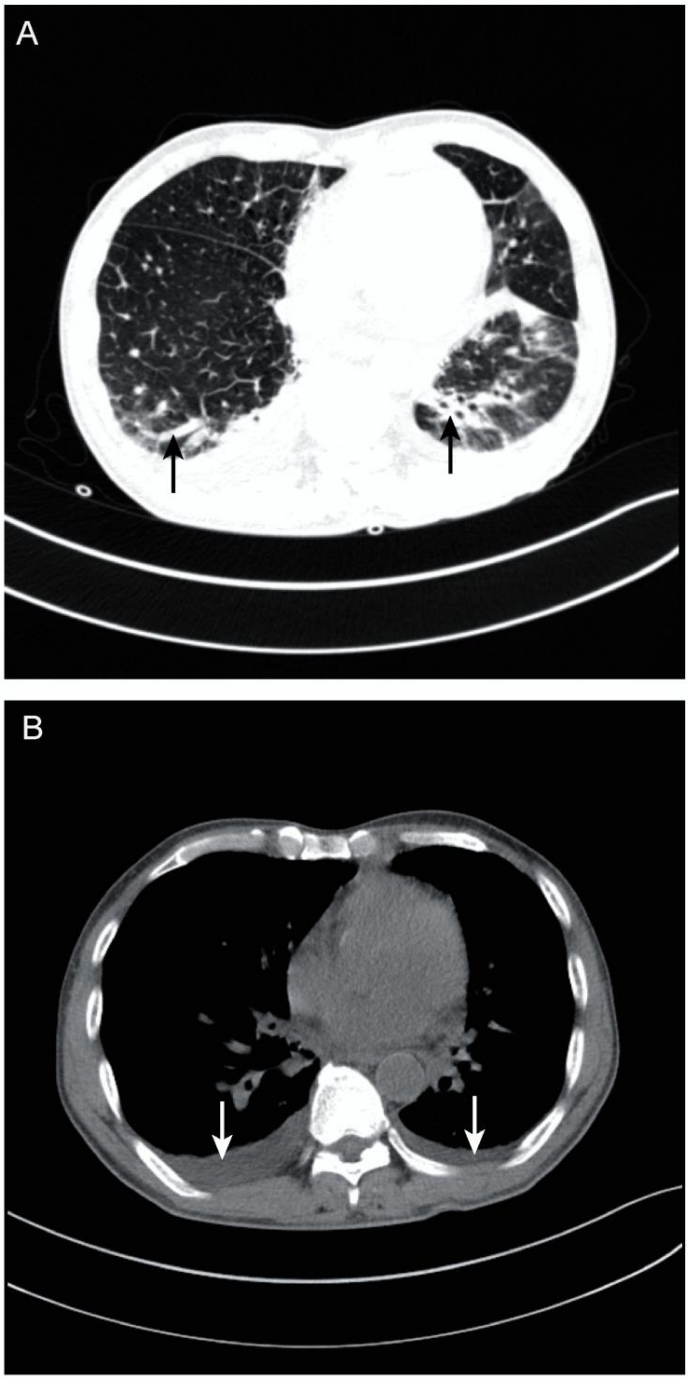

Technical Appendix Figure. The presence of pneumonia and hydrothorax in Patient 1 revealed by computerized tomography. Panel A showed exudative lesions on both lower lung; Panel B showed serous fluid on both pleural cavities.