

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

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.

References

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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 <i>Rickettsia</i>	<i>ompA</i> *	Rr190.70p	ATGGCGAATATTTCTCCAAAA	(1)
		Rr190.602n	AGTGCAGCATTGCTCCCCCT	
		190.70-38s1	AAAACCGCTTTATTACCC	(2)
		190.602-384r1	GGCAACAAGTTACCTCCT	
	<i>gltA</i> †	CS1d	ATGACTAATGGCAATAATAA	(3)
		CSEndr	CTTATACTCTCTATGTACA	
		RpCS877p	GGGGACCTGCTCACGGCGG	
<i>Anaplasma phagocytophilum</i>	<i>gltA</i>	RpCS1258n	ATTGCAAAAAGTACAGTGAACA	(4)
		W1	TGTTTTGGAGTGTGGAGAC	
		W1	GGTGAACCAATCTCAGCAA	
		N1	ATATAGAAAATCTGATCGG	
"A. capra"	<i>gltA</i>	N2	CTCTAAGTTTGCCTCAGC	(5)
		Outer-f	GCGATTTTAGAGTGYGGAGATTG	
		Outer-r	TACAATACCGGAGTAAAAGTCAA	
		Inner-f	GGGTTTCMTGTCTYACTGCTGCGTG	
<i>Babesia microti</i>	18S rRNA	Inner-r	TTGGATCGTARTTCTTGTAGACC	(6)
		Bab1	CTTAGTATAAGCTTTTATACAGC	
		Bab4	ATAGGTCAGAACTTGAATGATACA	
		Bab2	GTTATAGTTTATTTGATGTTTCGTTT	
severe fever with thrombocytopenia syndrome virus	S-segment	Bab3	AAGCCATGCGATTTCGCTAAT	(7)
		Forward	TTCACAGCAGCATGGAGAGG	
		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 *ompA* 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 *ompA* sequence of *R. helvetica* (GenBank accession number KU310588).

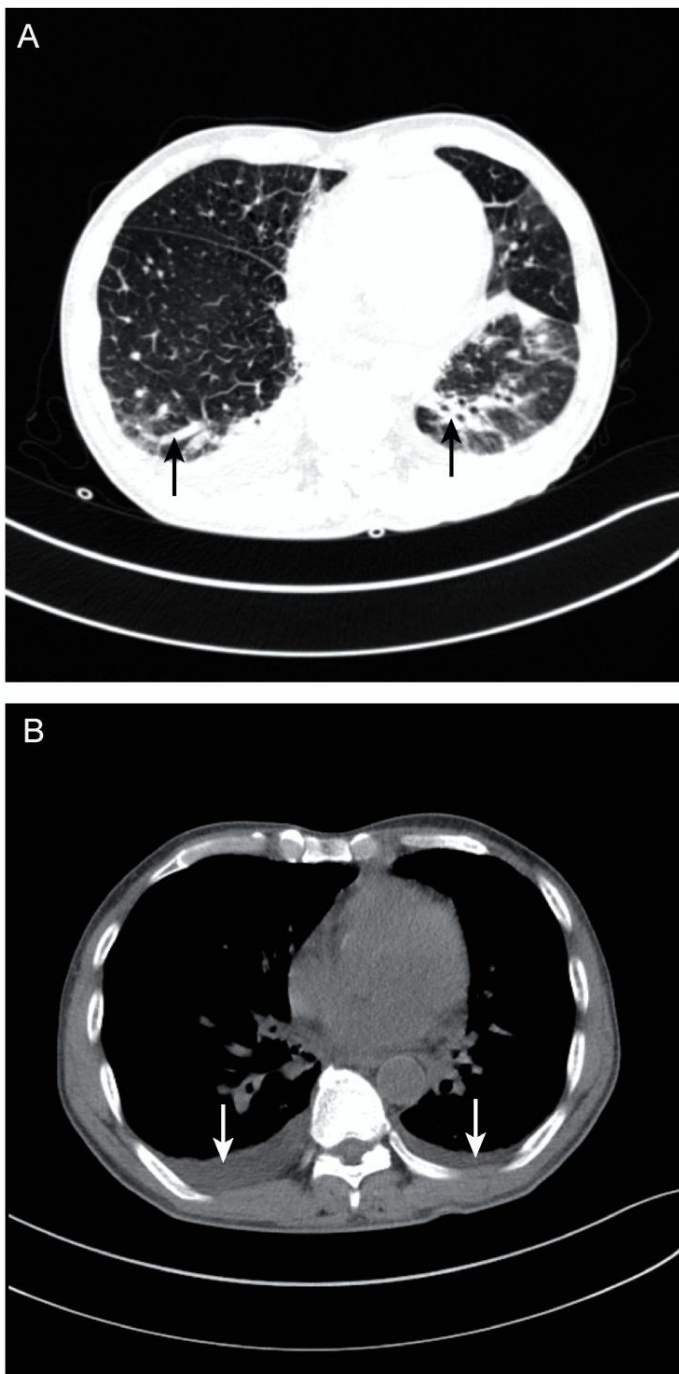
Technical Appendix Table 2. Sequence similarity of *ompA* and *gltA* genes between *Rickettsia* sp. XY99 and other *Rickettsia* strains

Species	Strain	Country	Host	<i>ompA</i>		<i>gltA</i>	
				GenBank accession number	Similarity	GenBank accession number	Similarity
<i>R. massiliae</i>	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	-	-
<i>R. rhipicephali</i>	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	-	-
<i>Rickettsia</i> sp. R300	R300	Brazil	Tick	AY472040	338/350	AY472038	1144/1150
<i>R. aeschlimannii</i>	MC16	Morocco	Tick	U43800	335/350	U59722	1142/1150
	Stavropol	Russia	Tick	DQ235777	336/350	DQ235776	1141/1150
<i>R. amblyommii</i>	Ac37	Brazil	Tick	CP012420	327/350	CP012420	1134/1150

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

Patient No.	Age	Sex	Days after onset		IFA*	
			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.



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.