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Isolation and Identification of *Rickettsia raoultii* in Human Cases: A Surveillance Study in 3 Medical Centers in China

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Background. *Rickettsia raoultii* is frequently detected in multiple tick species, whereas human infection remains scarcely studied.

Methods. A surveillance study was performed at 3 sentinel hospitals in China, to recruit participants with suspected tick exposure. *Rickettsia raoultii* infection was identified through polymerase chain reaction, followed by sequencing, and confirmed serologically. Isolation by cell culture was performed and the isolates were genome sequenced.

Results. Twenty-six subjects were determined to have *R. raoultii* infection, including 7 with asymptomatic infection, 15 with mild to moderate illness, and 4 with severe illness. Common nonspecific manifestations in the 19 patients with mild to moderate or severe illness included fever (100%), malaise (95%), myalgia (58%), lymphadenopathy (53%), and nausea (42%). Only 5% of them had rash, and 16% had eschar. Scalp eschar and neck lymphadenopathy after a tick bite syndrome was only seen in 2 patients. Of the 4 patients with severe complications, 3 developed pulmonary edema, and 1 developed clouding of consciousness and lethargy. Frequent abnormalities of laboratory testing included leukopenia, thrombocytopenia, lymphopenia, neutropenia, hypoproteinemia, and elevated levels of total bilirubin, hepatic aminotransferases, lactate dehydrogenase, and creatine kinase. All the 19 patients recovered without sequelae after receiving doxycycline treatment. Two *R. raoultii* strains were isolated, and a significantly less degraded genome was observed than other more virulent *Rickettsia* strains, indicating a low pathogenicity of the current strain.

Conclusions. Human infection with *R. raoultii* has a wide clinical spectrum that ranged from subclinical infection to severe complications. Physicians need to be aware of the high potential and clinical complexity of *R. raoultii* infection, to ensure appropriate testing and treatment in endemic regions.

Keywords. human infections; *Rickettsia*; spotted fever group rickettsiosis; ticks; China.

Spotted fever group (SFG) rickettsioses are caused by obligate intracellular bacteria and transmitted mainly with ticks [1, 2]. Three novel rickettsial genotypes (RpA4, DnS12, and DnS28) were detected in ticks from Russia in 1999 [3]. These agents were identified as a novel species of SFG rickettsiae through genotypic and phenotypic analysis, and named *Rickettsia raoultii* in 2008 [4]. Up to now, *R. raoultii* has been found in many European and Asian countries [4–8]. The main vectors were *Dermacentor* ticks (ie, *D. reticulatus*, *D. marginatus*, *D. nuttalli*, *D. silvarum*). Other hard ticks, such as *Haemaphysalis*, *Rhipicephalus*, *Hyalomma*, and *Amblyomma* ticks, were also involved.

Rickettsia raoultii was initially implicated in human infection in 2006 through detection of DNA in the blood of a Spanish patient [9]. The disease related to *R. raoultii* infection, called scalp eschar and neck lymphadenopathy after a tick bite (SENLAT), is characterized by a tick bite, an inoculation eschar on the scalp, and cervical lymphadenopathy [10]. Since then, 15 human cases of *R. raoultii* infection have been reported, including 4 confirmed cases identified by polymerase chain reaction (PCR) assay [9, 11, 12], 7 probable cases identified by serological testing [13–16], and 4 probable cases identified by PCR of the ticks harvested from the patients [14, 16].

In China, *R. raoultii* has been reported in 9 tick species, mainly from northern regions such as Jilin, Heilongjiang, Liaoning, Xinjiang Autonomous Region, and Inner Mongolia Autonomous Region. The prevalence in ticks ranged from 0.6% to 66.5% [17–21]. Because ticks act as both the reservoir and the vector of SFG rickettsiae, the geographical distribution of the disease is thought to be superposed on that of the ticks [2]. In contrast, only 2 cases have been reported in China [12]; the lack of information on human cases has largely caused delay in diagnosis and therapy. By performing hospital-based surveillance, we identified cases with *R. raoultii* infection featured by differential clinical manifestations as compared to previous reports. Two *R. raoultii* strains were isolated in human cases, and 1 isolate was genome sequenced.

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PATIENTS AND METHODS

Patients

A surveillance study was performed from 2015 to 2016 in 3 hospitals: the Hospital of Inner Mongolia Forest Branch of Chinese People's Armed Police Forces in Yakeshi City, the People's Liberation Army 154 hospital in Xinyang City, and the Affiliated Hospital of Taishan Medical University in Tai'an City (designated hospitals A, B, and C, respectively; Supplementary Appendix Figure 1). All 3 hospitals were located in regions with heavy tick activity and designated as medical institutions to treat patients with suspected tick-borne diseases. Subjects who had histories of tick bites, animal contact, or field activities within past 1 month, and visited a doctor at the 3 hospitals, were recruited into the study and screened for the infections of SFG rickettsiae.

All subjects had acute phase serum samples and whole blood samples collected before treatment was initiated. Convalescent phase serum samples were also collected beyond 14 days after disease onset. A standardized questionnaire was used to collect demographic data, medical history, and environmental exposure information, as well as the clinical information on symptoms and signs, laboratory tests, treatment, and outcome. The study was approved by the ethical committee of the Beijing Institute of Microbiology and Epidemiology, and all patients provided written informed consent to participate in this study.

Molecular Detection

DNA was extracted from ethylenediaminetetraacetic acid anticoagulated blood specimens collected at admission by using the QIAamp Blood Mini Kit (Qiagen). Nested PCR assays targeting the outer membrane protein A encoding gene (*omp*A), citrate synthase gene (*glt*A), and 17-kDa antigen-encoding gene were concurrently performed to detect the presence of SFG rickettsial DNA (Supplementary Appendix).

Rickettsial Isolation

Heparinized blood samples collected at admission were used for rickettsial isolation attempts by using cell culture. The buffy coat was separated after blood was left to sediment for 1 hour, and inoculated onto a semiconfluent monolayer of Vero cells in a T25 tissue culture flask containing 5 mL of Eagle's minimal essential medium with 5% fetal bovine serum. Cell cultures were incubated at 34.0°C in a 5% CO_2-enriched atmosphere. Giemsa staining and indirect immunofluorescence assay (IFA) were performed to detect the rickettsial growth. In case of positive examination, genomic DNA was extracted and used for amplifications of 2 panbacterial genes, 16S rRNA (*rrs*) and *glt*A, and 3 *Rickettsia*-specific genes, *omp*A, *omp*B, and PS120 encoding gene (*sca*4). For morphologic characterization, infected cells were processed for transmission electron microscopic examination with a CM-120 electron microscope (Philips).

Serologic Test

The IFA using the isolated *R. raoultii* strain as coating antigen was performed to test immunoglobulin G (IgG) antibody in the paired serum samples of the patients (Supplementary Appendix). Titer of 1:64 for IgG antibody was considered as positive reaction. The cutoff value was determined by testing the IFA slide on the known IgG- positive and -negative samples simultaneously. An acute infection of SFG rickettsiae was defined by seroconversion or a 4-fold increase in titers of IgG antibodies between acute and convalescent phase serum samples.

Amplicon Sequencing

PCR amplicons were purified, and then sequenced on a 3730 DNA Sequencer (Applied Biosystems). Genetic sequences were analyzed with ClusterW software (version 1.83). Phylogenetic analyses based on sequences of *rrs*, *glt*A, *omp*A, *omp*B, and *sca*4 were performed with the maximum likelihood method with MEGA software (version 5.0).

Whole-Genome Sequencing and Analyses

The genomic DNA of the current isolated *R. raoultii* strain was subject to high-throughput sequencing by using Illumina Miseq. A 600-bp insert length library was constructed by using Miseq Reagent Kit version 2. The software Newbler v2.9 was used for assembly with reference strain *R. raoultii* Khabarovsk [22]. The genome was annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline.

Genes for virulence of our strain were identified by BLAST search in the virulence factor database. The genomic comparison of our strain with other rickettsial strains was performed using the BLAST Ring Image Generator (BRIG; http://brig.sourceforge.net/). The Pan-Genomes Analysis Pipeline was used to search for orthologues among our strain genome and 22 well-documented *Rickettsia* genomes. The coding sequences (CDSs) of our strain were performed with BLAST search against the CDSs of other 3 strains (*Rickettsia slovaca* strain 13-B, *Rickettsia conorii* strain Malish7, and *R. rickettsii* strain Shelia Smith), to identify the orthologous, fragment, remnant, and absent genes.

RESULTS

Rickettsia raoultii Infection

From 2015 to 2016, a total of 1295 subjects, including 1089 patients and 206 asymptomatic subjects (Supplementary Appendix) were screened for the infections of SFG rickettsiae, and 26 (2.0%) were infected with *R. raoultii* according to PCR assays. In hospital A, 11 (3.9%) subjects were positive for amplifications, and the nucleotide sequences of *omp*A (308-bp), *glt*A (341-bp), and 17-kDa antigen-encoding gene (394-bp) (GenBank accession numbers KY474583, KY474584, and

KY474585, respectively) from these subjects were identical to each other and to the corresponding sequences of *R. raoultii* strain Khabarovsk (GenBank accession number CP010969) that was detected from *D. silvarum* ticks in Russian Far East. Two (1.6%) patients in hospital B and 13 (1.5%) in hospital C were positive for amplifications, and the *omp*A, *glt*A, and 17-kDa antigen-encoding gene sequences (GenBank accession numbers KY474580, KY474581, and KY474582) were identical to each other, showing 99.4%, 99.7%, and 100% similarities, respectively, to those of *R. raoultii* strain Khabarovsk.

Two stable *Rickettsia* isolates were obtained from 2 febrile patients in hospital A who had adequate samples for isolation. No positive culture was obtained from the 2 patients in hospital B or 13 patients in hospital C. After 12 days of incubation, intracellular and free *Rickettsia*-like organisms were observed using Giemsa staining and IFA (Figure 1). Phylogenetic analyses based on nucleotide sequences of *rrs* (1368-bp), *glt*A (1070-bp), *omp*A (509-bp), *omp*B (512-bp), and *sca*4 (2920-bp) showed that the 2 isolates were identical (designated strain IM-16) and had 100% similarity to *R. raoultii* strain Khabarovsk (Supplementary Appendix Figure 2). Observation of *R. raoultii* isolates in Vero cells with transmission electron microscopy showed that rickettsiae were located within the cytoplasm and appeared to be rod-shaped or coccoid bacteria that had an electron-lucent halo adjacent to the microcapsular layer and cell wall (Figure 1).

Four of the 11 subjects in hospital A had available paired samples, among whom 3 were seroconverted to *R. raoultii*, and 1 showed a 4-fold increase of the IgG antibody titer by IFA (Table 1). Both patients in hospital B showed a 4-fold increase of the IgG antibody titer. All 13 patients in hospital C had serological evidence of infection, with seroconversion in 9 and a 4-fold increase of the IgG antibody titer in 4.

Epidemiologic and Clinical Characteristics

Seven of the 26 (27%) subjects were asymptomatic at the time of visiting the doctor, but with concern of disease due to confirmed tick bites. Their median age was 24 years (range, 21–28 years), and all were men. All 7 subjects had reported recent tick bite and field activity, and 1 (14%) had also reported wild animal contact. The 7 asymptomatic subjects received no antibiotic treatment and made no revisit to the hospital. The follow-up telephone inquiry confirmed that none of them developed disease related to the tick bites.

Nineteen of the 26 (73%) subjects had mild to moderate or severe illness. Their median age was 43 years (range, 20–76 years), and 13 (68%) were men. Reported possible tick exposure within past 1 month included field activity in 18 (95%) patients, tick bite in 7 (37%), and animal contact in 4 (21%). The median time from tick bite to the onset of illness was 4 days (range, 3–5 days) and from illness onset to visit a doctor was 3 days (range, 2–7 days), respectively.

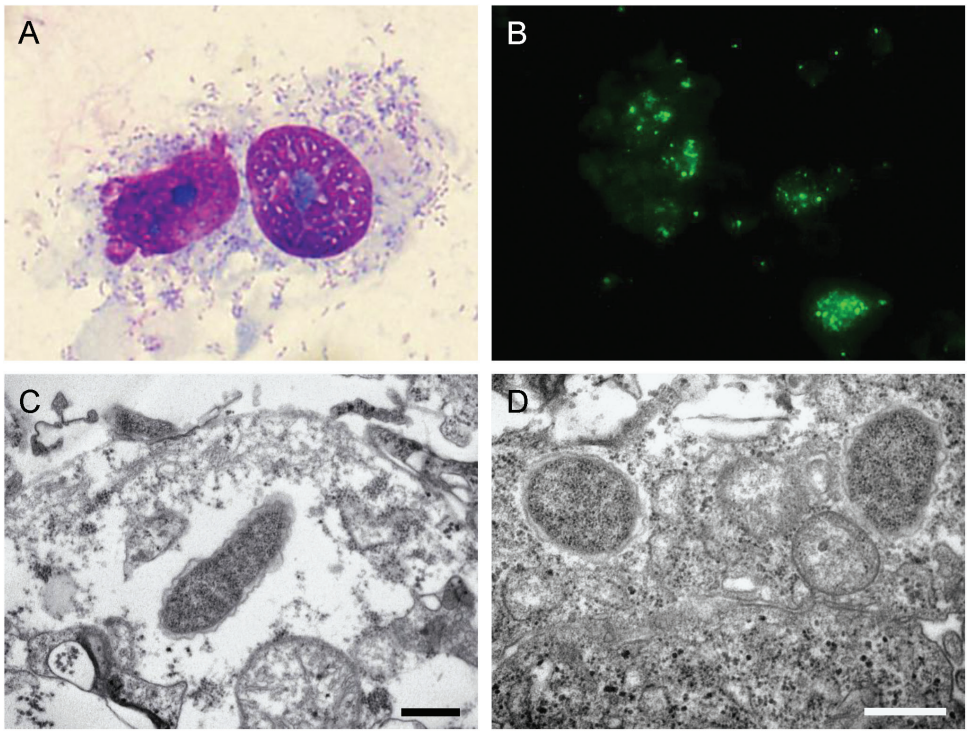

Figure 1. Photomicrographs of cells infected with *Rickettsia raoultii*. *A*, Giemsa-stained Vero cells. *B*, Agent grown in Vero cells and detected on immunofluorescence assay of a convalescent serum sample obtained from a patient. *C* and *D*, Electron photomicrographs of Vero cells infected with *R. raoultii*.

Table 1. Serum Immunoglobulin G Antibody Titers of the Subjects With *Rickettsia raoultii* Infection

Subject No.[a]	Age, y	Sex	Time of Sample Collection[b]		IFA[c]	
			AP	CP	AP	CP
1	21	Male	1	NA	<64	NA
2	22	Male	2	NA	<64	NA
3	23	Male	1	NA	<64	NA
4	24	Male	1	NA	<64	NA
5	25	Male	3	NA	<64	NA
6	28	Male	2	NA	<64	NA
7	27	Male	2	NA	<64	NA
8	20	Male	2	16	<64	512
9[d]	24	Male	3	17	<64	128
10	20	Male	2	16	64	256
11[d]	26	Male	3	17	<64	64
12	35	Female	4	17	64	256
13	38	Male	4	16	64	256
14	62	Male	2	18	<64	512
15	50	Male	3	18	<64	512
16	59	Male	5	20	64	512
17	43	Female	7	25	128	512
18	44	Female	6	19	64	256
19	46	Female	5	19	<64	256
20	56	Female	5	16	64	256
21	45	Male	6	14	<64	128
22	50	Male	3	15	<64	128
23	69	Male	4	16	<64	128
24	66	Male	2	14	<64	64
25	76	Female	3	15	<64	64
26	74	Male	3	14	<64	64

Abbreviations: AP, acute phase; CP, convalescent phase; IFA, immunofluorescence assay; NA, not available.

[a]Subjects 1–11 were from hospital A; subjects 12–13 were from hospital B; and subjects 14–26 were from hospital C.

[b]Number of days after the onset of illness for patients in the acute phase or the convalescent phase of the illness.

[c]Data are the reciprocals of the serum dilution.

[d]The *Rickettsia raoultii* strains were cultured from subjects 9 and 11.

All the 19 patients had illnesses, and nonspecific manifestations included fever in all (range of the highest body temperature, 37.8°C –40.0°C), malaise in 18 (95%), myalgia in 11 (58%), headache in 4 (21%), cough in 3 (16%), and chills in 2 (11%) (Table 2). Gastrointestinal symptoms were reported, with nausea in 8 (42%) patients, vomiting in 3 (16%), and diarrhea in 3 (16%). Only 1 (5%) patient had rash and 3 (16%) had eschar. Lymphadenopathy was observed in 10 (53%) patients, with 6 presenting on groins, 2 presenting on armpits, and 2 presenting on neck. Ecchymosis around venipuncture site was reported in 2 (11%) patients, and face edema was reported in 1 (5%) patient. SENLAT syndrome was only seen in 2 patients. Four (21%) patients presented with severe complications, including 3 with pulmonary edema and 1 with clouding of consciousness and lethargy. Three of the patients had preexisting conditions before admission into the hospital, including pulmonary tuberculosis in patient 23, chronic obstructive pulmonary disease in patient 25, and viral hepatitis type B in patient 26. Patient 24 was found to be coinfected with *Klebsiella pneumoniae* by culture of sputum sample collected at admission.

For the 15 patients with mild or moderate illness, hemogram test results showed leukopenia in 5 (33%), thrombocytopenia in 8 (53%), lymphopenia in 1 (7%), and neutropenia in 4 (27%). While the 4 patients with severe illness had more frequent abnormalities, with leukopenia in 3 (75%), thrombocytopenia in 4 (100%), lymphopenia in 2 (50%), and neutropenia in 3 (75%) (Table 2). Blood biochemistry test results showed that 4 of the 19 (21%) patients had hypoproteinemia and 3 (16%) had increased levels of total bilirubin. Of the 15 patients with mild or moderate illness, slightly increased levels of hepatic aminotransferases, lactate dehydrogenase (LDH), and creatine kinase (CK) were observed in 5 (33%), 4 (27%), and 2 (13%) of the patients, respectively. In contrast, all 4 patients with severe illness had remarkably increased levels of hepatic aminotransferases (>100 U/L) and LDH (>400 U/L), and 2 of them had remarkably increased CK level (>400 U/L).

Table 2. Clinical Characteristics and Laboratory Test Results of the Patients With *Rickettsia raoultii* Infection

Characteristic	Patients With *Rickettsia raoultii* Infection (n = 19)	Patients With Mild or Moderate Illness (n = 15)	Patients With Severe Illness (n = 4)
Clinical signs			
Fever	19 (100)	15 (100)	4 (100)
Highest temperature, °C, median (range)	38.7 (37.8–40.0)	38.5 (37.8–39.6)	39.4 (39.0–40.0)
Headache	4 (21)	3 (20)	1 (25)
Face edema	1 (5)	1 (7)	0
Malaise	18 (95)	14 (93)	4 (100)
Myalgia	11 (58)	7 (47)	4 (100)
Chills	2 (11)	0	2 (50)
Cough	3 (16)	0	3 (75)
Nausea	8 (42)	5 (30)	3 (75)
Vomiting	3 (16)	3 (20)	0
Diarrhea	3 (16)	3 (20)	0
Rash	1 (5)	0	1 (25)
Eschar	3 (16)	3 (20)	0
Lymphadenopathy	10 (53)	8 (53)	2 (50)
Ecchymosis	2 (11)	1 (7)	1 (25)
Pulmonary edema	3 (16)	0	3 (75)
Lethargy	1 (5)	0	1 (25)
Clouding of consciousness	1 (5)	0	1 (25)
Laboratory findings[a]			
Leukopenia	8 (42)	5 (33)	3 (75)
Thrombocytopenia	12 (63)	8 (53)	4 (100)
Lymphopenia	3 (16)	1 (7)	2 (50)
Neutropenia	7 (37)	4 (27)	3 (75)
Hypoalbuminemia	4 (21)	2 (13)	2 (50)
Increased total bilirubin	3 (16)	2 (13)	1 (25)
High ALT or AST concentration			
40–100 U/L	5 (26)	5 (33)	0
>100 U/L	4 (21)	0	4 (100)
High LDH concentration			
245–400 U/L	4 (21)	4 (27)	0
>400 U/L	4 (21)	0	4 (100)
High CK concentration			
200–400 U/L	3 (16)	2 (13)	1 (25)
>400 U/L	2 (11)	0	2 (50)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase.

[a]Normal ranges: for leukocyte count, 4.0–10.0 cells × 10^9/L; for platelets, 150–300 cells × 10^9/L; for lymphocytes, 0.4–4.4 cells × 10^9/L; for neutrophils, 1.7–7.7 cells × 10^9/L; for albumin, 35.0–55.0 g/L; for total bilirubin, 5.1–17.1 μmol/L; for ALT, 0–40 U/L; for AST, 0–40 U/L; for LDH, 109–245 U/L; for CK, 25–200 U/L.

Six of the 19 patients were outpatients. They had oral doxycycline treatment at home with 100 mg twice daily for 2–4 days, and reported no clinically significant sequelae when they revisited the hospital 14 days later. The remaining 13 inpatients received doxycycline treatment with 100 mg twice daily for 3–7 days until fever disappeared and clinical manifestations resolved. Seven of the 13 inpatients were initially treated with other antimicrobial agents, including cefoperazone in 3 and levofloxacin in 4. When the patients were discharged from hospital, mild laboratory abnormalities persisted in 3 patients, including thrombocytopenia in 2 patients, increased hepatic aminotransferase levels in 2 patients, increased LDH level in 2 patients, and increased CK level in 1 patient.

Genomic Features of the *R. raoultii* Strain

Rickettsia raoultii strain IM16 genome yields a G+C content of 32.5%. A total of 1520 CDSs, 3 ribosomal RNAs (rRNAs) (16S, 23S, and 5S rRNA), 3 noncoding RNAs, and 33 transfer RNAs were predicted (Supplementary Appendix Figure 3). Of all the 1528 CDSs, 48.1% exhibit forward strain (GenBank accession number CP019435). Genes for virulence in *R. raoultii* strain IM16 include *adr1*, *adr2*, *tlyC*, *pat1*, *pat2*, *pld*, *rickA*, *ompA*, *ompB*, *sca2*, *sca4*, *virB2*, *virB3*, *virB4* (2 copies), *virB6* (5 copies), *virB8* (2 copies), *virB9* (2 copies), *virB10*, *virB411*, and *virD4* (Supplementary Appendix).

BRIG analysis revealed that *R. raoultii* had 6 distinctive genomic regions compared to other *Rickettsia* species (Supplementary Appendix Figure 4). A total of 424 core genes

were identified when 23 *Rickettsia* genomes were taken into analysis. The core genes showed a wide range of functional categories, mainly involving the categories representing catalytic activity, cellular process, and metabolic process (Supplementary Appendix). Phylogenetic tree based on the concatenated nucleotide sequences of the core genes generated a reliable delineation of evolutionary relationships across *Rickettsia* species and resulted in 7 clusters (Supplementary Appendix Figure 5). *Rickettsia raoultii* had the near relationships with *R. montanensis*, *R. massiliae*, *R. rhipicephali*, and *R. amblyommii*, and these 5 *Rickettsia* species comprised cluster B. In total, 275 single-nucleotide polymorphisms of the 424 core genes (423 642 bp) were identified between *R. raoultii* strain IM16 and strain Khabarovsk, indicating a low genomic diversity.

The genome comparison was performed to explore the gene degradation in relationship with the virulence. *R. raoultii* strain IM16 had additional 41, 21, and 12 protein-coding regions more than *R. rickettsii* strain Shelia Smith, *R. conorii* strain Malish7, and *R. slovaca* strain 13-B, respectively. *Rickettsia raoultii* strain IM16 sequentially shared 1218, 1192, and 1183 orthologous open reading frames with the 3 *Rickettsia* species (Table 3). When compared by the number of degraded genes (split, remnant, and absent), *R. raoultii* strain IM16 had a significantly less degraded genome than *R. rickettsii* strain Shelia Smith (119 vs 223, $P < .001$), *R. conorii* strain Malish7 (114 vs 231, $P < .001$), and *R. slovaca* strain 13-B (130 vs 212, $P < .001$).

DISCUSSION

In the current study, we have identified *R. raoultii* infection in 19 patients and 7 apparently healthy subjects by using molecular and serologic methods. They were from 3 regions that host different ecosystems and predominant tick species, that is, forestry regions mainly infested with *D. nuttalli* and *Ixodes persulcatus* in northeastern vs humid regions that host *Haemaphysalis longicornis* in eastern central China. All the 3 tick species have been detected to carry *R. raoultii*, also with a wide geographic distribution in China mainland, possibly indicating a wider distribution of *R. raoultii* in endemic regions. In addition, our isolation of *R. raoultii* from the patients provide definitive evidence that *R. raoultii* is a human pathogen.

Before the current research, a total of 15 human cases of *R. raoultii* infection had been reported, including 4 confirmed cases and 11 probable cases. Of the 4 confirmed cases, 1 had SENLAT syndrome, 2 had rash, and 1 had suspected tick-borne disease [9–12]. All 11 probable cases had SENLAT syndrome [13–16]. In the present study, the *R. raoultii* infection caused asymptomatic or mild to moderate illness. Common clinical features were unspecific, including fever, malaise, myalgia, and gastrointestinal symptoms. Lymphadenopathy was also frequently seen in our patients; however, SENLAT syndrome was observed in only 2 patients. This is in contrast to the *R. raoultii* infection in European patients, from whom SENLAT was considered as the exclusive feature to make differential diagnosis [9, 10, 23]. The difference in clinical features might be associated with the location of tick bites [12] and the virulence of the endemic strains.

From the clinical perspective, the current *R. raoultii* might be less pathogenic than other SFG rickettsiae, such as *R. rickettsii* strain Shelia Smith (the agent of Rocky Mountain spotted fever) and *R. conorii* strain Malish7 (the agent of Mediterranean spotted fever) [2, 24]. Indeed, *R. slovaca* that also caused SENLAT was found to be more virulent than *R. raoultii* [16, 22]. In the current study, we first obtained the genome sequence from a *R. raoultii* strain of human origin and further explored the genetic features that might underlie the strain's virulence. A reliable and precise evolutionary relationship was obtained by phylogenetic analysis based on the concatenated 424 core genes, revealing that *R. raoultii*, together with *R. montanensis*, *R. massiliae*, *R.*

Table 3. Number of Open Reading Frames Diversely Conserved in *Rickettsia raoultii* Strain IM16 in Comparison With 3 More Virulent Rickettsiae[a]

	Orthologous ORFs With *Rickettsia raoultii* IM16 (1520)			*Rickettsia raoultii* IM16 ORFs in Excess			Reference ORFs in Excess		
Reference	Best Match	Split in IM16[b]	Split in Reference[c]	Fragment	Remnant in Reference	Absent in Reference	Fragment	Remnant in IM16	Absent in IM16
Rickettsia slovaca 13-B (1508)	1144	34 (16)	110 (40)	130	61	111	124	59	55
Rickettsia conorii Malish7 (1499)	1119	26 (10)	122 (47)	144	61	123	134	54	60
Rickettsia rickettsii Shelia Smith (1479)	1113	27 (11)	113 (43)	157	65	115	134	51	57

Abbreviation: ORF, open reading frame.

[a]The coding sequences (CDSs) of our strain were performed with BLAST search against the CDSs of another 3 strains (*R. slovaca* strain 13-B, *R. conorii* strain Malish7, and *R. rickettsii* strain Shelia Smith). CDSs that shared at least 70% identity and 70% coverage were signed orthologous genes. The remnant and fragment genes were characterized by a homemade script. Genes that shared no similarities against the reference genome were signed absent genes.

[b]Numbers in parentheses indicate the orthologous open reading frames in the reference genome.

[c]Number in the bracket indicates the orthologous ORFs in *R. raoultii* str. IM16 genome.

rhipicephali, and *R. amblyommii*, comprised a cluster, which was thought to cause mild disease or considered to be nonpathogenic. The *R. raoultii* strain IM16 genome includes the common virulence factors conserved among SFG rickettsiae, suggesting a minor role of the virulence genes in determining its pathogenicity. However, when the gene contents were compared to more virulent *R. slovaca*, *R. conorii*, and *R. rickettsii*, the current *R. raoultii* strain IM16 was shown to have more intact genes. We propose that some of the genes may play important roles in maintaining a low virulence level, as gene loss has been thought to be involved in the development of pathogenicity [25, 26].

It is noteworthy that 4 patients in our study presented with severe illness, such as pulmonary edema, neurologic symptoms, and multiorgan dysfunction. The factors, such as older ages, underlying immune-related conditions, and coinfections with other bacteria, might contribute to the development of these complications. Indeed, clinical physicians should be aware of these patients with these risk factors, to avoid adverse disease outcome.

For laboratory findings of *R. raoultii* infection, leukopenia, thrombocytopenia, neutropenia, and elevated levels of hepatic aminotransferases, LDH, and CK, were neither unspecific. This has made the clinically differential diagnosis with viral infection such as the endemic severe fever with thrombocytopenia syndrome virus infection difficult [27]. Although laboratory methods such as serologic test by IFA, PCR, and immune detection of tissue are effective diagnostic tools for rickettsial infection [28, 29], these technologies are still unavailable in rural hospitals in China [30]. It is presumed that *R. raoultii* might be largely misdiagnosed or underdiagnosed, especially in rural residents who had higher risk for tick exposure.

In conclusion, we identified that *R. raoultii* infection was associated with a wide clinical spectrum ranging from asymptomatic infection to mild illness and even severe complications. Public health workers and physicians need to be aware of the wide distribution and clinical complexity of *R. raoultii* infection. Further genetic analysis based on more strains might lead to an enhanced understanding of the pathogenicity of this *Rickettsia* species.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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