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Rickettsia japonica infections in Huanggang, China, in 2021

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ABSTRACT

Two patients from Huanggang, China, were diagnosed with spotted fever group (SFG) rickettsiosis—caused by *spotted fever group rickettsiae* (*SFGR*)—in 2021. This study aimed to investigate the clinical symptoms, laboratory examinations, epidemiological factors, and therapeutic responses in patients with SFG rickettsiosis—an emerging disease in this region. The patients showed a variety of clinical signs and symptoms, such as acute febrile illness with severe headache, myalgia, asthenia, anorexia, eschar, lymphadenopathy, and rash on the trunk and extremities. They exhibited increased neutrophil ratio, mild thrombocytopenia, liver dysfunction, and increased C-reactive protein and procalcitonin levels. Following treatment with doxycycline, the patients recovered completely.

This is the first report of *Rickettsia japonica* infection in Huanggang City, Hubei Province, China. *SFGR* infection is a tick-borne disease, which can be effectively treated with doxycycline; however, it has a mortality rate of approximately 10% with delays in treatment. The Huanggang area is also a high-risk area for tick-borne severe fever with thrombocytopenia syndrome (SFTS). Therefore, SFTS and SFG rickettsiosis should be carefully diagnosed in this area and clinicians should be alert with respect to the possibility of infections with both SFTS and SFG rickettsiosis.

Introduction

Spotted fever group rickettsiae (*SFGR*) contains more than 20 species of *rickettsiae* that are pathogenic to humans and distributed worldwide. In recent years, cases of spotted fever group (SFG) rickettsiosis have been reported in Jiangsu, Jiangxi, Henan, Anhui, and Shandong provinces and the Inner Mongolia Autonomous Region in China [1–5]. Some cases were diagnosed by routine monitoring of the target population in forested regions, while several cases were confirmed by retrospective detection of *Rickettsia* in the serum of patients with febrile illnesses [1,2]. Public health experts who monitored wild mammals and ticks in China found a variety of *SFGR* that can cause human diseases, indicating that several regions in China could be natural foci [4,6]. However, relatively few clinical cases of SFG rickettsiosis have been reported in hospitals in these areas. *Rickettsiae* are gram-negative intracellular bacteria and may not be detected using nucleic acid tests due to false negative results, especially when rickettsial antibodies appear late. There are some similarities and differences among the clinical features of the disease found in different regions. These factors may lead to misdiagnosis and delays in initiation of treatment. SFG rickettsiosis has not been previously reported in Hubei Province, China where Haemaphysalis longicornis is widely distributed. The onset of illness for the two patients were highly consistent in time and space, indicating that the pathogen may have spread to the local population. The purpose of this study was to investigate the epidemiological characteristics, clinical manifestations, laboratory results, and treatment responses of patients with SFG rickettsiosis in this region. The findings of the study could be used as a basis to formulate prevention and treatment plans for the disease.

Case description

Two patients from Fuzihe Town, Macheng City, Huanggang City, Hubei Province, which is a hill area, presented to the Department of Infectious Diseases of Union Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology in August 2021 with fever, fatigue, severe headache, and rash. One is a 62 year old male and the other is a 59 year old female. They all lived in hill region.

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2214-2509/

Laboratory tests

Peripheral blood samples were obtained from each patient according to standard procedures during admission, and procalcitonin (PCT) levels, C-reactive protein (CRP) levels, D-dimer, urinary sediment microscopy and differential blood cell counts were examined at the Clinical Diagnosis Laboratory of the Union Hospital. Plasma levels of creatinine, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were determined at the Biochemistry Laboratory of the Union Hospital. Furthermore, the activated partial thromboplastin time and thrombin time of fresh plasma samples were measured at the Clinical Diagnosis Laboratory of the Union Hospital.

The patients had failed to respond to antiviral and antibacterial therapy in the primary hospital during the initial stage of illness. After admission to our hospital, we performed quantitative metagenomics next-generation sequencing (mNGS) tests. For mNGS tests, blood samples were transported to the molecular lab of Hangzhou Matridx Biotechnology Co., Ltd., within 24 h.

Library preparation and sequencing

Whole blood was centrifuged at 1600 g for 10 min and the supernatant was further centrifuged at 16,000*g* for 10 min to separate the plasma. For cerebrospinal fluid, DNA or RNA sequencing was performed. DNA or RNA sequencing libraries were prepared by reverse transcription (for RNA), enzymatic fragmentation (except for plasma), end repair, terminal adenylation, and adapter ligation (NGSmaster™ library preparation, Matridx, Cat# MAR002) [7]. The concentration of libraries was quantified by real-time PCR (KAPA) and pooled. Shotgun sequencing was carried out on the Illumina NextSeq platform. Approximately 20 million 75 bp single-end reads were generated for each library. For each run, one negative control (artificial plasma mixed with fragmented human genomic DNA) and one positive control (a mixture of inactivated bacteria, fungi and pseudoviral particles containing synthesized DNA or RNA fragments of *adenovirus* and *influenza A virus*, respectively) were included for quality control.

Bioinformatics pipeline

Raw sequencing data were analyzed using a bioinformatics pipeline, which included the following steps: (1) Unnecessary adapter sequences and low-quality bases (Q-score cut-off of 20) were trimmed. (2) Human host sequences were eliminated by mapping to the human reference genome (GRCh38.p13) using BWA (Burrows-Wheeler alignment, http://bio-bwa.sourceforge.net). (3) After removal of low-complexity reads, the remaining sequencing data were simultaneously aligned by BWA to the reference databases (NCBI nt database and GenBank) to identify microbial species.

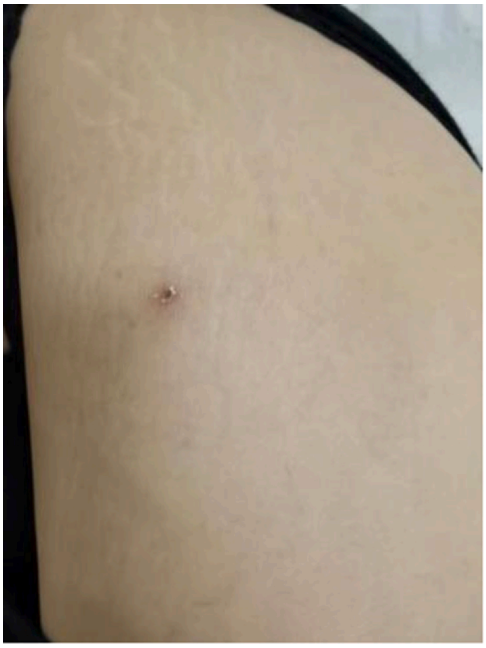

Fig. 2. Eschar visible on the skin of the lateral surface of the right thigh of patient 2.

Clinical findings and laboratory examination

Both patients reported history of fieldwork, and one had a known tick bite. Both patients had fever, asthenia, severe headache, and anorexia and displayed lymphadenopathy and characteristic rash manifested as dark reddish spots (Fig. 1a,b). The characteristic rash of patient 1 transformed into ecchymosis (Fig. 1c) in convalescence stage. (Fig. 1). Patient 2 had eschars (Fig. 2), but neither of them had any severe complications (i.e. respiratory failure; or hemorrhagic or neurological signs or symptoms) (Table 1). None of their household contacts exhibited similar symptoms.

Peripheral blood examination in both patients suggested lymphopenia, eosinopenia, thrombocytopenia, and nomal WBC levels, but the ratio of neutrophils was increased. The inflammatory indicators, including D-dimer, PCT, and CRP, were significantly increased. Biochemical examination showed that the levels of liver transaminase and lactate dehydrogenase increased upon admission (Table 2).

After 3–5 days of doxycycline treatment, the clinical symptoms disappeared, and laboratory test results returned to normal.

Diagnosis of patients with SFG Rickettsiosis

The pathogens detected using mNGS are listed in Table 3. In addition to common human symbiotic microorganisms, *spotted fever*

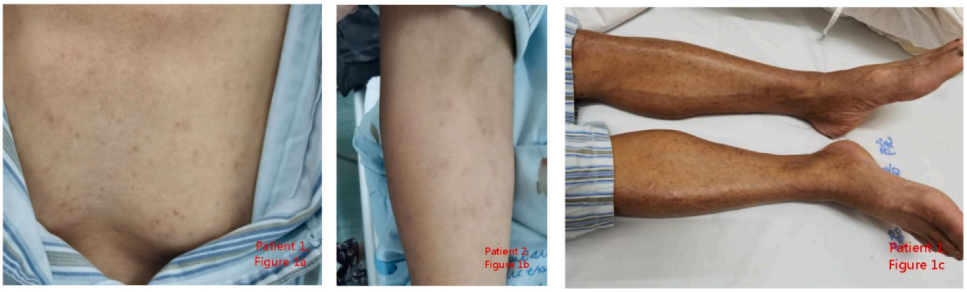

Fig. 1. A dark reddish, non-blanching, maculopapular rash was visible on the trunk and extremities of the patients (a, b). The characteristic rash of patient 1 transformed into ecchymosis (c) in convalescence stage.

Table 1
Epidemiologic and clinical characteristics of the two patients.

Characteristics	Patient no.	
	1	2
Age, y	62	59
Sex	M	F
History of tick bite	No	Yes
Field work	Yes	Yes
Time from tick bite to disease onset, d	NA	2
Time from disease onset to hospital admission, d	7	7
No. days hospitalization	9	7
Signs and symptoms		
Fever	Yes	Yes
Highest temperature, °C	39.1	40.2
Headache	Yes	Yes
Asthenia	Yes	Yes
Masseter weakness	Yes	No
Myalgia	No	Yes
Anorexia	Yes	Yes
Nausea	No	No
Cough	No	No
Confusion	No	No
Meningeal irritation sign	No	No
Ecchymosis	No	No
Rash	Yes	Yes
Eschar	No	Yes
Lymphadenopathy	Yes	Yes
Hematuria	Yes	No

rickettsiae and *herpes virus* were detected. A small amount of *Epstein-Barr virus* was detected in one patient, and a small amount of human *herpes virus* types 1 and 5 was detected in the other patient. Six 50 bp *Rickettsia* fragments from the two patients were aligned with the reference database, and the results showed that all six fragments were consistent with the gene library of *Rickettsia japonica* (Fig. 3a, b). Due to the small number of fragments detected, the whole gene sequence analysis could not be carried out.

Therapeutic responses

After treatment with doxycycline, the patient's conditions rapidly alleviated without sequelae.

Table 2
Laboratory test results of the two patients on admission.

Result	Normal range	Patient no.	
		1	2
WBC count ($\times 10^9$/l)	3.5–9.5	7.53	6.78
N%	40–75	74.6	86.5
E%	0.4–8.0	0	0
L%	20–50	17.6	12.2
Thrombocytopenia ($\times 10^9$/l)	125–350	89	113
Proteinuria	negative	positive	negative
Hematuria	negative	positive	negative
Total Bilirubin (μmol/l)	5.1–19.0	7.7	9.6
APTT (s)	28.0–43.5	26.5	32.9
ALT (U/l)	5–40	95	41
AST (U/l)	8–40	99	29
LDH (U/l)	109–245	498	319
D-dimer (mg/l)	< 0.5	4.51	2.32
PCT (μg/l)	< 0.5	12.2	0.68
CRP (mg/l)	< 8.00	105.0	83.1
Cr (μmol/l)	44.0–133.0	49.8	86.2

WBC count, white blood cell count; N%, neutrophil ratio; E%, Eosinophil ratio; L%, Lymphocyte ratio; APTT, activated partial thromboplastin time, ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PCT, procalcitonin; CRP, C-reactive protein; Cr, creatinine.

Table 3
Pathogenic microorganisms detected by mNGS in blood (sorted by species).

Patient no.	Name	Genus name	Genus reads accum
1	Staphylococcus	Staphylococcus	4
	Staphylococcus aureus	Staphylococcus	4
	Staphylococcus capitis	Staphylococcus	4
	Rickettsia	Rickettsia	2
	Spotted fever group	Rickettsia	2
	Cutibacterium	Cutibacterium	1
	Cutibacterium acnes	Cutibacterium	1
	Herpesviridae	Herpesviridae	14
	Gammaherpesvirinae	Gammaherpesvirinae	14
	Lymphocryptovirus	Lymphocryptovirus	14
	Human gammaherpesvirus 4 (Epstein-Barr virus)	Lymphocryptovirus	14
2	Moraxella	Moraxella	18
	Moraxella osloensis	Moraxella	18
	Cutibacterium	Cutibacterium	10
	Cutibacterium acnes	Cutibacterium	10
	Rickettsia	Rickettsia	4
	Spotted fever group	Rickettsia	4
	Staphylococcus	Staphylococcus	2
	Staphylococcus aureus	Staphylococcus	2
	Staphylococcus capitis	Staphylococcus	2
	Malassezia	Malassezia	1
	Malassezia restricta	Malassezia	1
	Herpesviridae	Herpesviridae	2
	Alphaherpesvirinae	Alphaherpesvirinae	1
	Simplexvirus	Simplexvirus	1
	Human alphaherpesvirus 1	Simplexvirus	1
	Betaherpesvirinae	Betaherpesvirinae	1
	Cytomegalovirus	Cytomegalovirus	1
	Human betaherpesvirus 5 (cytomegalovirus)	Cytomegalovirus	1

Discussion

This is the first report of an *SFGR* infection in Huanggang City, Hubei Province. We used the second-generation sequencing method to detect the gene fragment of the *SFG rickettsiae* subgenus in the blood of two febrile patients from the same township in the Huanggang area of Hubei Province and found that the infections were caused by *Rickettsia japonica* by gene comparison. The patients presented with marked fever, rash, headache, and one patient also reported myalgia. They exhibited nomal WBC levels, increased neutrophil ratio, eosinopenia, thrombocytopenia, significantly increased PCT and CRP, and slightly elevated transaminase and lactate dehydrogenase levels. The absence of severe complications in these two patients may be due to their low rickettsial load.

A small number of gene fragments from a variety of *herpes viruses* were detected in the blood of these two patients. These *herpes viruses* cause common latent infections in humans. As a natural pathogen, *Rickettsia japonica* can over activate macrophages and T lymphocytes [8]. This leads to an immune imbalance and a subsequent loss of immune control over latent *herpes viruses* in cells resulting in a small amount of nucleic acid being detectable in the blood. However, the primary symptoms in both patients were not consistent with *herpes virus* infection, and *herpes virus* infection is not responsive to doxycycline.

In 2013, *Rickettsia japonica* bacteria were detected in a patient and Haemaphysalis longicornis tick, and the subsequent epidemiological investigation and serological tests revealed that 54.8% of 902 healthy people living in rural areas of Anhui Province tested positive for *Rickettsia japonica* specific antibodies [3]. The two patients we reported to reside in the same township. However, this is the first

(a)

Query= NB502082:141:HVK37BGXJ:4:12602:7074:8950|601139159
1:N:0:TGGCTAAC+GTTAGCCA

Length=50

Sequences producing significant alignments:	Score (Bits)	E Value
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CP032049.1 Rickettsia japonica strain LA4/2015 chromosome, comple...	93.5	3e-16
AP017601.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017598.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017597.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017596.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017599.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017588.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017587.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017600.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017585.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017584.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017583.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017582.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017579.1 Rickettsia japonica DNA, complete genome, strain: OHH-1	93.5	3e-16
AP017577.1 Rickettsia japonica DNA, complete genome, strain: MZ08014	93.5	3e-16
AP017602.1 Rickettsia japonica DNA, complete genome, strain: YH_M	93.5	3e-16
AP017575.1 Rickettsia japonica DNA, complete genome, strain: HH07124	93.5	3e-16
AP017590.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
AP019862.1 Rickettsia heilongjiangensis CH8-1 DNA, complete genome	93.5	3e-16
CP003319.1 Rickettsia massiliae str. AZT80, complete genome	87.9	1e-14
CP015012.1 Rickettsia amblyommatis isolate An13, complete genome	87.9	1e-14

Query= NB502082:141:HVK37BGXJ:4:12602:7074:8950|601139159
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Length=50

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AP017601.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017594.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017586.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017585.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017584.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017583.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017582.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017579.1 Rickettsia japonica DNA, complete genome, strain: OHH-1	93.5	3e-16
AP017577.1 Rickettsia japonica DNA, complete genome, strain: MZ08014	93.5	3e-16
AP017602.1 Rickettsia japonica DNA, complete genome, strain: YH_M	93.5	3e-16
AP017575.1 Rickettsia japonica DNA, complete genome, strain: HH07124	93.5	3e-16
AP017590.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
AP019862.1 Rickettsia heilongjiangensis CH8-1 DNA, complete genome	93.5	3e-16
CP003319.1 Rickettsia massiliae str. AZT80, complete genome	87.9	1e-14
CP015012.1 Rickettsia amblyommatis isolate An13, complete genome	87.9	1e-14

Fig. 3. a: Blast Comparison results of the Detected SFGR 50 bp sequences of patient 1. b: Blast Comparison results of the Detected SFGR 50 bp sequences of patient 2.

report of SFG rickettsiosis in Hubei; it is possible that the disease has been transmitted to the local area and neighboring cities and counties. The prevalence of the disease needs to be clarified by epidemiological investigations and serological testing in the area. It would be useful if microbial experts studied pathogenic *Rickettsia* in more detail.

The correct identification and treatment of rickettsiosis would avoid unnecessary mortality and overuse of beta-lactam antimicrobials, which are ineffective against *Rickettsia* infections but contribute to the emerging problem of antimicrobial resistance. Unfortunately, besides the classic symptom triad, that is, fever, rash, and headache, it has been shown that each case of SFG rickettsiosis is characterized by specific features, including variations in the severity of symptoms. Furthermore, typical symptoms may be absent or overlooked [9]. Some patients infected with *Rickettsia japonica* exhibit no rash, and some do not complain of headache [1]. Therefore, the diagnosis of rickettsioses is a major challenge for physicians in regions where the disease is emerging [10]. There are several diagnostic methods available to identify SFG rickettsioses, including serological methods, cell cultures, and molecular methods. However, unless it is an severe infection, the level of bacteria in blood is lower than that in tissue samples from rash or eschars [11], nucleic acid testing of skin rash and eschar tissue has not yet been a routine test in primary hospital clinics; therefore, the detection of nucleic acid of *SFGR* in blood may be false negative. Antibodies are detected only seven to 15 days after the occurrence of the disease [12], therefore, serological tests are also not sensitive in the early stage of an acute illness when most patients look for medical help. The isolation of *SFGR* is very difficult and is generally only carried out in scientific research institutions. These factors have caused great difficulties in the timely diagnosis and treatment of patients in primary hospitals. Notably, the Huanggang area is also an epidemic area of the SFTS.

Haemaphysalis longicornis ticks carry *Rickettsia japonica*, *Candidatus Rickettsia longicornii, Candidatus Rickettsia jiaonani, Anaplasma phagocytophilum, Ehrlichia,* and *severe fever with thrombocytopenia syndrome virus* [13,14]. Haemaphysalis longicornis ticks are widely distributed in the hilly areas of Huanggang City and other cities in the Huaiyangshan mountain region of Hubei and Henan, China, and are active from April to October every year. Patients can be infected with the aforementioned pathogens by Haemaphysalis longicornis ticks bites when picking tea, weeding, or planting vegetables.

Patients with both SFTS and SFG rickettsiosis may have fever, headache, lymphadenopathy, thrombocytopenia and eosinophilia in the acute stage, and hence, the diseases are easily misdiagnosed. Doxycycline is an ineffective treatment for SFTS; however, severe illness or death may occur if SFG rickettsiosis is not swiftly treated with effective antibiotics. A retrospective investigation in an *SFTS virus*-endemic region of China identified *SFTS virus-SFGR* co-infection in approximately 8.5% of *SFTS virus*-infected patients and a higher frequency of fatal outcomes and delayed recuperation in the co-infected patients [15]. The study found that patients co-infected with SFTS and SFG rickettsiosis were not diagnosed at the time of treatment and did not receive doxycycline. This could be because the symptoms of SFTS were more pronounced and the degree of platelet reduction and transaminase elevation are milder in SFG rickettsiosis than in SFTS [16]. Therefore, some of the characteristic symptoms of SFG rickettsiosis were not recognized. Although patients with SFG rickettsiosis have increased PCT and CRP levels, which is very different from SFTS in early stage, physicians often consider this to be due to secondary bacterial and fungal infections with significantly reduced granulocytes by *SFTS-virus*. Hence, physicians prescribe antibacterial and antifungal drugs therapeutically or prophylactically at that time, and they are less likely to choose doxycycline or clarithromycin.

We recommend the use of mNGS for diagnosing patients with fever of unknown origin in natural foci where Haemaphysalis longicornis is present, to allow timely detection and treatment of SFG rickettsiosis, especially in the early epidemic stage in this region. To reduce medical costs when the number of affected patients increases, clinicians should summarize the clinical characteristics of SFG rickettsiosis in this area, and provide reference data for early diagnosis and treatment of local patients with SFG rickettsiosis. Although the laboratory diagnosis of SFG rickettsiosis is difficult, in the areas where Haemaphysalis longicornis is present, physicians in primary hospitals need to improve their awareness of SFG rickettsiosis, take the disease into account when treating patients with

(b)

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Length=50

Sequences producing significant alignments:	Score (Bits)	E Value
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AP017597.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017596.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
AP019862.1 Rickettsia heilongjiangensis CH8-1 DNA, complete genome	93.5	3e-16
CP003375.1 Rickettsia slovaca str. D-CWPP, complete genome	87.9	1e-14
CP003341.1 Rickettsia parkeri str. Portsmouth, complete genome	87.9	1e-14
CP003319.1 Rickettsia massiliae str. AZT80, complete genome	87.9	1e-14
EF215912.1 Rickettsia rickettsii isolate AZ-8 BioY (bioY) gene, p...	87.9	1e-14
EF215911.1 Rickettsia rickettsii isolate Hauke BioY (bioY) gene, ...	87.9	1e-14
CP018914.1 Rickettsia rickettsii strain Iowa isolate Small Clone,...	87.9	1e-14
CP003318.1 Rickettsia rickettsii str. Hauke, complete genome	87.9	1e-14
AY345069.1 Rickettsia conorii strain URRCCroatia29 RC0779 folC-RC...	87.9	1e-14

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AP017598.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017597.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017596.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017595.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017593.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017592.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017599.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017588.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017594.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017587.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017600.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017586.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017585.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017584.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017583.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017582.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017579.1 Rickettsia japonica DNA, complete genome, strain: OHH-1	93.5	3e-16
AP017577.1 Rickettsia japonica DNA, complete genome, strain: MZ08014	93.5	3e-16
AP017602.1 Rickettsia japonica DNA, complete genome, strain: YH_M	93.5	3e-16
AP017575.1 Rickettsia japonica DNA, complete genome, strain: HH07124	93.5	3e-16
AP017590.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
AP019862.1 Rickettsia heilongjiangensis CH8-1 DNA, complete genome	93.5	3e-16
CP003375.1 Rickettsia slovaca str. D-CWPP, complete genome	87.9	1e-14
CP003341.1 Rickettsia parkeri str. Portsmouth, complete genome	87.9	1e-14
CP003319.1 Rickettsia massiliae str. AZT80, complete genome	87.9	1e-14
EF215912.1 Rickettsia rickettsii isolate AZ-8 BioY (bioY) gene, p...	87.9	1e-14
EF215911.1 Rickettsia rickettsii isolate Hauke BioY (bioY) gene, ...	87.9	1e-14
CP018914.1 Rickettsia rickettsii strain Iowa isolate Small Clone,...	87.9	1e-14
CP003318.1 Rickettsia rickettsii str. Hauke, complete genome	87.9	1e-14
AY345069.1 Rickettsia conorii strain URRCCroatia29 RC0779 folC-RC...	87.9	1e-14

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Length=50

Sequences producing significant alignments:	Score (Bits)	E Value
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CP032049.1 Rickettsia japonica strain LA4/2015 chromosome, comple...	93.5	3e-16
AP017601.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017598.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017597.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017596.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017595.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017593.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017592.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017599.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017588.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017594.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017587.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017585.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017582.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017579.1 Rickettsia japonica DNA, complete genome, strain: OHH-1	93.5	3e-16
AP017577.1 Rickettsia japonica DNA, complete genome, strain: MZ08014	93.5	3e-16
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AP017575.1 Rickettsia japonica DNA, complete genome, strain: HH07124	93.5	3e-16
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AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
AP019862.1 Rickettsia heilongjiangensis CH8-1 DNA, complete genome	93.5	3e-16
CP003375.1 Rickettsia slovaca str. D-CWPP, complete genome	87.9	1e-14
CP003341.1 Rickettsia parkeri str. Portsmouth, complete genome	87.9	1e-14
CP015012.1 Rickettsia amblyommatis isolate An13, complete genome	86.1	5e-14
CP003319.1 Rickettsia massiliae str. AZT80, complete genome	82.4	6e-13
CP018914.1 Rickettsia rickettsii strain Iowa isolate Small Clone,...	80.5	2e-12
CP003318.1 Rickettsia rickettsii str. Hauke, complete genome	80.5	2e-12

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1:N:0:ACCCCAGG+CCTGGGGT

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AP017596.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
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AP017582.1 Rickettsia japonica DNA, nearly complete genome, strai...	93.5	3e-16
AP017579.1 Rickettsia japonica DNA, complete genome, strain: OHH-1	93.5	3e-16
AP017577.1 Rickettsia japonica DNA, complete genome, strain: MZ08014	93.5	3e-16
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AP017580.1 Rickettsia japonica DNA, complete genome, strain: PO-1	93.5	3e-16
AP017572.1 Rickettsia japonica DNA, complete genome, strain: DT-1	93.5	3e-16
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CP018914.1 Rickettsia rickettsii strain Iowa isolate Small Clone,...	80.5	2e-12
CP003318.1 Rickettsia rickettsii str. Hauke, complete genome	80.5	2e-12

Fig. 3. (*continued*)

headache, fever, mottled rash, and eschar, and use effective antimicrobial drugs to greatly reduce the risk of *SFGR* infections. They must be alert to signs of rash and eschar, and levels of PCT and CRP when treating patients with SFTS. Doxycycline should also be used empirically in patients with SFTS with no significant relief of symptoms or with severe headache.

Ethical approval

Written informed consent was obtained from patients. In our setting, ethical approval for case reports are not required if informed consent is given.

Acknowledgement

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Conflict of interest

No conflict of interest to declare.

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