

## Case Report

# Japanese spotted fever complicated with pleural effusion in Zhejiang province, China: a case report and literature review

Keting He<sup>1#</sup>, Shangci Chen<sup>1#</sup>, Liang Yu<sup>1#</sup>, Dongfan Wei<sup>2</sup>, Xiaowei Xu<sup>1</sup>

<sup>1</sup> State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

<sup>2</sup> Department of Dermatology, Affiliated Hangzhou Dermatology Hospital, Zhejiang University School of Medicine, Hangzhou, China

# Authors contributed equally to this work.

### Abstract

**Introduction:** Japanese spotted fever (JSF) mainly occurs in Japan; however, it has been increasingly reported in China. JSF is typically characterized by fever, rash, and eschar, in addition to non-specific symptoms. Yet, reports on the pulmonary indicators in JSF are limited. Herein, we report an unusual case of JSF associated with pleural effusion and pneumonia, in which the pathogen was identified via blood next-generation sequencing (NGS).

**Case presentation:** We report a case of a 33-year-old woman who presented with fever for five days, rash for two days, and myalgia, fatigue, and edema for one day. She had recently been on vacation when an unknown insect bit her. The doctors at the local primary hospital considered a bacterial infection and administered dexamethasone, ceftriaxone, indomethacin, and anti-allergy agents, but the symptoms persisted. A rash without pruritus or pain developed gradually over the entire body and face. We considered rickettsial infection and administered doxycycline and levofloxacin. Metagenomic NGS from blood confirmed the presence of *Rickettsia japonica* (*R. japonica*). Abdominal computed tomography revealed bilateral pleural effusion with two atelectasis; patchy shadows with blurred edges, and uniform enhancement in both lower lungs. After several days of treatment, the symptoms and laboratory results improved.

A literature review of the epidemiology of *R. japonica* and JSF in China, characteristics of JSF, and related pulmonary changes, and technology to diagnose JSF is provided.

**Conclusions:** JSF has a variety of symptoms and is becoming increasingly popular in China. Clinical doctors need to identify it carefully.

**Key words:** Japanese spotted fever; next-generation sequencing; pleural effusion; *Rickettsia japonica*; *Rickettsia pneumonia*.

*J Infect Dev Ctries* 2024; 18(7):1135-1140. doi:10.3855/jidc.18354

(Received 11 April 2023 – Accepted 14 November 2023)

Copyright © 2024 He *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Introduction

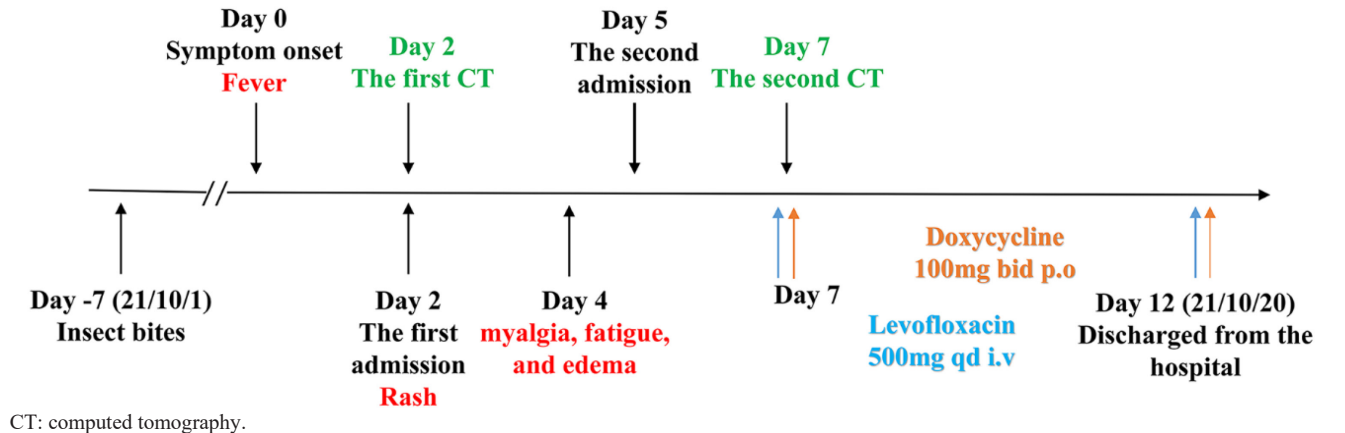
Japanese spotted fever (JSF) is an infectious disease caused by the pathogen *Rickettsia japonica* (*R. japonica*). JSF was first discovered in 1984 and reported in 1985 by Japanese doctors [1]. Generally, JSF is considered a tick-borne disease, but land leeches are also potential *R. japonica* vectors [2]. JSF is prevalent mainly in Japan, where 1765 cases were reported from May 2007 to January 2016 via national surveillance [3]; although mortality data are limited. However, JSF has also been reported in China [4], South Korea [5], and Thailand [6]. Here, we report the case of a symptomatic young woman with next-generation sequencing (NGS)-confirmed JSF in China.

### Case presentation

A 33-year-old previously healthy woman visited the emergency room of our hospital on October 12, 2021. She had a fever for five days, rash for two days, and myalgia, fatigue, and edema for one day. She had been on vacation in a mountainous region of Jinhua, Zhejiang Province, China, seven days before the onset of symptoms (Figure 1). On the way home, she noticed a prick on her right inner thigh along with two bites, which spontaneously resolved.

Initially, she had a fever (38–39 °C) and chills, and she took ibuprofen. However, the symptoms persisted, with fever reaching 40.6 °C. She went to a local primary hospital on the third day of symptoms. By this time, she had scattered rash on her forearms and trunk, which was

**Figure 1.** The patient’s clinical course depicted as a timeline, showing the occurrence, progression, and treatment of Japanese spotted fever.



not accompanied by itching or pain. Chest computed tomography (CT) at the local hospital indicated bilateral pulmonary micro-nodules and a few fibrous lesions in the right middle lobe. Unfortunately, we did not have access to those images. She was then diagnosed with a bacterial infection and treated with dexamethasone, ceftriaxone, indomethacin, and anti-allergy agents; but the specifics of this treatment were not available to us.

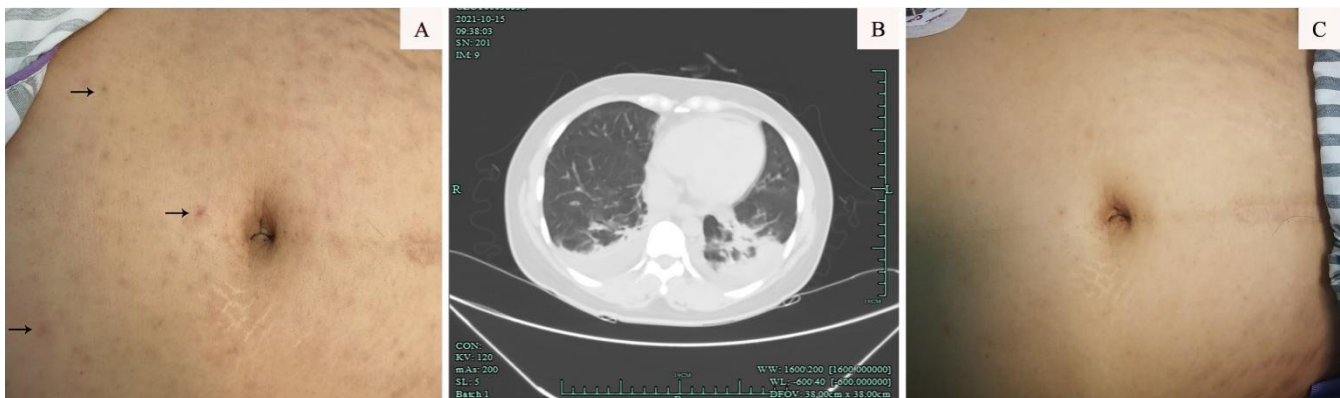
Nevertheless, that therapy did not improve the patient’s condition, and she developed severe myalgia, edema, and fatigue. Hence, the patient was admitted to our hospital on the fifth day of illness. On admission, assessment of vital signs showed high body temperature (39.7 °C), hypotension (85/49 mmHg), tachycardia (131 beats per minute), tachypnea (20 breaths per minute), and normal oxygen saturation (99%). Numerous red rashes, which were no more than 1 cm in diameter and without pruritus or tenderness, spread over her body and face. In addition, no eschar was observed (Figure 2A).

Laboratory tests revealed a normal white blood cell count, mild anemia, low platelet count, high C-reactive protein (CRP) and procalcitonin levels, hypoalbuminemia, mildly elevated alanine aminotransferase and aspartate aminotransferase levels, mild coagulation dysfunction, elevated D-dimer levels, and mild electrolyte disturbances. The levels of cytokines, including interleukin-6 (IL-6), IL-10, and interferon-gamma (IFN-γ), were significantly increased (Table 1). The erythrocyte sedimentation rate and immunoglobulin (Ig) E levels were normal.

On the seventh day of illness, an abdominal CT scan at our hospital revealed bilateral pleural effusion with two atelectases, patchy shadows with blurred edges, and uniform enhancement in both lungs (Figure 2B), together with hepatosplenomegaly. Ultrasonography revealed multiple bilateral axillary and inguinal lymphadenopathies.

Considering the clinical manifestations and travel history, we determined that the patient had a rickettsial infection. Therefore, we administered doxycycline (100

**Figure 2.** Patient characteristics before and after treatment. (A) On admission, numerous red rashes of no more than 1 cm in diameter without pruritus or tenderness had spread over the patient’s body and face. (B) An abdominal computed tomography exam shows bilateral pleural effusion with two atelectasis and patchy shadows with blurred edges and uniform enhancement in both lungs. (C) After treatment, the rash subsided.



mg twice a day) and levofloxacin (500 mg once a day) to fight the infection and dexamethasone (5 mg once) to reduce the inflammation. Liver-protecting agents, albumin, vitamins, potassium chloride, and calamine lotion were administered as supplements. Finally, the pathological agent was identified by NGS from blood samples, showing that *R. japonica* was the most likely causative agent, with a relative abundance of 20.4% and an identification confidence of 99%.

The patient's body temperature dropped, the rash resolved (Figure 2C), and laboratory tests improved (Table 1) after the appropriate treatment. The patient was discharged on the eighth day after admission. To date, she has had no sequelae of JSF.

## Literature review

### *R. japonica* and JSF in China

Approximately 50 JSF cases have been reported in China, mainly in the eastern and southern regions [4,7-11]. The male to female ratio was approximately 1:1, and most patients were middle-aged or older adults. Severe cases accounted for 25%, and the mortality rate was 3.8%.

However, the prevalence of *R. japonica* and its distribution in China are not very clear. A study of *Rickettsia* species in *Haemaphysalis longicornis* (*H. longicornis*) ticks in Shandong Province, Eastern China, revealed that the ticks were infected with multiple *Rickettsia* species, including *R. japonica* [12]. Hedgehogs are a host for ticks and are thought to play an important role in the transmission of various tick-borne pathogens (TBPs). One study detected TBPs in ticks found on hedgehogs from Jiangsu Province, eastern China. All the hedgehogs were *Erinaceus amurensis* and most ticks were *Haemaphysalis flava* (*H. flava*). In addition, *H. longicornis* were also identified. The results showed that *R. japonica* can be detected in most *H. flava* (81.1%) ticks, and hedgehogs may contribute to the natural cycles of *R. japonica* [13]. Li

*et al.* found that 54.8% (494/902) of serum samples from rural healthy adults were positive for *R. japonica*-specific antibodies in the Anhui Province, China [8]. Out of 34 provinces and special administrative regions of China, 10 areas (29.4%) were *R. japonica*-positive and four provinces had human infections [7]. These studies suggest that the pathogen is more widespread than previously thought.

### General characteristics of JSF and related pulmonary changes

JSF progresses rapidly and the disease's typical triad includes high fever, rash, and eschar. The rash tends to start on the limbs and quickly spreads throughout the body, particularly on the palms and soles. Rashes are usually not accompanied by itching or pain. Other common clinical features include fatigue, gastrointestinal symptoms, myalgia, headache, tachycardia, rapid breathing, hypotension, edema, and purpura. However, a small number of patients experience disseminated intravascular coagulation [14], respiratory failure [15], central nervous system infection [14], arthritis [16], multiorgan failure [17], ventricular tachycardia [18], and shock; fatal cases have also been reported [19]. Laboratory tests usually show low platelet counts; hypoproteinemia; and elevated liver enzymes including bilirubin, creatinine kinase, blood urea nitrogen, creatinine, and CRP levels [20,21]. In addition, serum levels of cytokines [e.g., tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, and IFN- $\gamma$ ] and chemokines [e.g., IL-8, interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ), and MIP-1 $\beta$ ] are significantly increased in the acute phases of JSF. Thus, the severity of JSF may be related to hypercytokinemia [22]. Additionally, fatal cases of JSF show several characteristics compared to non-fatal cases including old age; less frequently recorded fever and eschar; higher frequency of severe signs, such as

**Table 1.** Laboratory test results.

Laboratory tests	Day 4	Day 6	Day 11	Reference range
White blood cells ( $\times 10^9/L$ )	8.5	5.64	12.29	4–10
Platelets ( $\times 10^9/L$ )	122	96	226	101–320
Hemoglobin (g/L)	110	98	107	113–151
Neutrophils (%)	88.3	88.9	43.2	50–70
CRP (mg/L)	58.02	114	12.4	0.0–8.0
PCT (ng/mL)	-	1.58	0.18	0.00–0.50
Total protein (g/L)	-	53	61.8	65–85
ALT (U/L)	-	42	90	7–40
AST (U/L)	-	65	90	13–35
IL-6 (pg/mL)	-	1494.28	17.7	0.10–2.92
IL-10 (pg/mL)	-	61.1	1.45	0.1–5
IFN- $\gamma$ (pg/mL)	-	1036.66	0.65	0.10–2.90

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRP: C-reactive protein; IFN: interferon; IL: interleukin; PCT: procalcitonin.

liver dysfunction and disseminated intravascular coagulation (DIC) [3]. Studies on JSF sequelae are rare, as most studies concentrate on conditions during hospitalization and most severe cases can recover after effective treatment. Approximately 2.5% of patients will suffer from sequelae, including peripheral neuropathy, cutaneous necrosis secondary to acute infectious purpura fulminans, deafness, and cerebral infarction [23]. A study in China reported that patients with JSF suffered from purpura fulminans and required amputation or skin grafting. Several patients had intracranial hemorrhage, deep venous thrombosis, acute cerebral infarction, or arrhythmia, even in mild patients. Although the study did not mention the long-term prognosis of these patients, it revealed the risks of sequelae and consequent disability in patients with JSF [10]. The severe complications mentioned above also suggest the probability of sequelae.

Clinical manifestations of diseases result from the underlying pathogenic mechanisms and their effects. In rickettsial disease, the main pathological change is vasculitis. *Rickettsia* spp. spread throughout the organs via the bloodstream and mainly infects endothelial cells, thereby increasing vascular permeability, which explains the edema, hypotension, and reduced organ perfusion. Some severe cases can develop interstitial pneumonia, noncardiogenic pulmonary edema, and diffuse alveolar damage in the lungs [24]. *Rickettsia* also induces cell signaling cascades in endothelial cells, causing the secretion of cytokines and chemokines; thus, endothelial cells display pro-inflammatory properties. Additionally, the activation of innate and acquired immunity also contribute to changes in cytokines [24,25]. Different types of rickettsial infections lead to different cytokine profiles, and these cytokines have varied functions. In some types of rickettsial infections, IL-1 $\beta$  and type 1 immune cytokines can account for protective inflammatory response [26]. IL-8 and IL-6 can predict the severe infection outcome with a significant level of cell death [27]. There is a lack of studies exploring the association between specific cytokines and clinical outcomes in the case of JSF.

Reports on JSF with pulmonary changes are limited. Matsuura described a patient with JSF who had a cough and progressive dyspnea. A chest CT scan revealed bilateral interstitial infiltrate, ground-glass opacities, interlobular septal thickening, and consolidation. Finally, the patient was diagnosed with rickettsial pneumonia [28]. A recent article also reported patients with JSF having multiple effusions, including pleural effusion [7]. Rickettsiosis cases, such as scrub typhus, murine typhus, human monocytic ehrlichiosis,

Mediterranean spotted fever, and Israeli spotted fever, can be accompanied by pleural effusion [29]. In the present case, pleural effusion and pulmonary lesions were observed in a matter of days. Since doxycycline treatment was effective, we speculate that they may be related to JSF; although pulmonary symptoms were not as evident during the imaging. In addition, the patient's chest CT could be regarded as community-acquired pneumonia, which might lead to confusing interpretations. Thus, atypical etiological agents should not be excluded, especially when patients do not respond well to usual treatments.

#### *Common technologies to detect Rickettsia*

Recent developments in technology have made it possible to choose common laboratory diagnostic methods to detect various pathogens. Serology is simple, fast, and often inexpensive. Therefore, it is still commonly used worldwide. For *Rickettsia* spp., the serological methods include indirect immunofluorescence assays (IFAs), enzyme-linked immunosorbent assay (ELISA), and the Weil-Felix test. The sensitivity and specificity of different serological methods have been compared [30]. However, serology has some drawbacks. For instance, the result may be disturbed by cross-reactions, and standard cutoff values to prevent false positives and negatives are lacking. Despite this, IFA is usually considered the gold standard for the laboratory diagnosis of *Rickettsia* spp. infections [31]. In addition, the sensitivity of serological methods depends on the timing of blood collection from the patient, and obtaining meaningful results before IgM appears is difficult. Therefore, these methods are not sensitive in the acute phase. Thus, it is recommended to compare serum antibody levels in the acute and convalescent phases [32].

Molecular detection methods, such as polymerase chain reaction (PCR), are widely used for diagnosing infectious diseases. PCR is fast and sensitive in the acute phase, which compensates for the limitations of serological testing [33]. PCR can test different types of samples, such as blood, eschar, pus, and effusions. For JSF, the eschar PCR positivity rate was higher than that of blood PCR (88.2% vs. 45.3%) [21]. Thus, testing diverse samples could increase the diagnostic rate.

Culturing etiological agents is also common in clinical practice and allows the testing of different types of specimens. For optimal sensitivity, specimens should be obtained as soon as possible or before antibiotic administration. However, it often takes several days to obtain the results. Moreover, biosafety concerns must be considered due to their infectivity, and related

processes should be performed in a biosafety level 3 laboratory [33].

Sequence analyses, including NGS, are becoming increasingly important for the study of infectious diseases. Metagenomic NGS (mNGS) was used in this case, as it is considered an unbiased approach to detect pathogens, which enables broad identification of known and unexpected organisms, and has the potential to also identify unknown organisms [34]. In contrast, PCR or serological tests can only detect specifically targeted organisms. Moreover, mNGS can provide precise species information. In this case, although we hypothesized that the patient was infected with *Rickettsia*, we could not confirm that the pathogen was *R. japonica*, as we had no prior experience with it.

However, mNGS also has disadvantages. In patient samples, the majority of reads (> 99%) are from the human hosts, which decreases the sensitivity of this technology because microbial non-human reads are relatively scarce. Targeted sequencing or host depletion methods can partially alleviate this problem [35]. Another drawback is that microbial contaminants complicate the analysis of results; therefore, microbes or nucleic acids in testing environments should be minimized. In addition, this technology is expensive, and the cost of testing should be considered.

### Limitations

This study has some limitations. First, we did not have access to the patient's initial chest CT scan (performed in another hospital). Thus, we could not compare or assess pulmonary lesions. Second, the patient had few pulmonary symptoms and we did not detect pathogens in pleural effusion or other respiratory tract samples; thus, we only speculated about a relationship between JSF and pulmonary lesions, based on her travel history, CT report comparisons, and therapeutic effects.

### Conclusions

*R. japonica* is more widespread in China than previously thought and is increasingly associated with pleural effusion. Pulmonary changes in JSF can be confused with those from community-acquired pneumonia; therefore, atypical pathogens such as *Rickettsia* spp. should be considered when conventional antibiotics do not work well. In addition, a JSF diagnosis requires detailed medical history, careful physical examination, and appropriate diagnostic methods that doctors must master. In particular, NGS is becoming increasingly relevant for diagnosing infectious diseases, especially in non-endemic areas.

### Ethics approval and informed consent

This report was approved by the First Affiliated Hospital, Zhejiang University School of Medicine's ethics committee, who provided consent to publish the case details. Written informed consent for publication of the clinical details and clinical images was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal.

### Consent for publication

All authors confirm that they had full access to all the data in the study and accept responsibility for the publication. The patient also gave consent for publication.

### Acknowledgments

This work was supported by the Natural Science Foundation of Zhejiang Province (LQ21H190004). We would like to thank Editage (www.editage.cn) for their English language editing.

### References

- Mahara F, Koga K, Sawada S, Taniguchi T, Shigemi F, Suto T, Tsuboi Y, Ooya A, Koyama H, Uchiyama T (1985) The first report of the rickettsial infections of spotted fever group in Japan: three clinical cases. *Kansenshogaku Zasshi* 59: 1165–1171. doi: 10.11150/kansenshogakuzasshi1970.59.1165.
- Sando E, Suzuki M, Katayama M, Taira M, Fujita H, Ariyoshi K (2019) *Rickettsia japonica* infection after land leech bite, Japan. *Emerg Infect Dis* 25: 1243–1245. doi: 10.3201/eid2506.181985.
- Kinoshita H, Arima Y, Shigematsu M, Sunagawa T, Saijo M, Oishi K, Ando S (2021) Descriptive epidemiology of rickettsial infections in Japan: scrub typhus and Japanese spotted fever, 2007–2016. *Int J Infect Dis* 105: 560–566. doi: 10.1016/j.ijid.2021.02.069.
- Li H, Zhang PH, Du J, Yang ZD, Cui N, Xing B, Zhang XA, Liu W (2019) *Rickettsia japonica* infections in humans, Xinyang, China, 2014–2017. *Emerg Infect Dis* 25: 1719–1722. doi: 10.3201/eid2509.171421.
- Chung MH, Lee SH, Kim MJ, Lee JH, Kim ES, Lee JS, Kim MK, Park MY, Kang JS (2006) Japanese spotted fever, South Korea. *Emerg Infect Dis* 12: 1122–1124. doi: 10.3201/eid1207.051372.
- Gaywee J, Sunyakumthorn P, Rodkvamtook W, Ruang-arereate T, Mason CJ, Sirisopana N (2007) Human infection with *Rickettsia* sp. related to *R. japonica*, Thailand. *Emerg Infect Dis* 13: 657–659. doi: 10.3201/eid1304.060585.
- Teng ZQ, Gong P, Wang W, Zhao N, Jin XJ, Sun XR, Zhou HJ, Lu JL, Lin XB, Wen BH, Kan B, Xu JG, Qin T (2023) Clinical forms of Japanese spotted fever from case-series study, Zigui county, Hubei province, China, 2021. *Emerg Infect Dis* 29: 202–206. doi: 10.3201/eid2901.220639.
- Li J, Hu W, Wu T, Li HB, Hu W, Sun Y, Chen Z, Shi Y, Zong J, Latif A, Wang L, Yu L, Yu X-J, Liu B-Y, Liu Y (2018) Japanese spotted fever in Eastern China, 2013. *Emerg Infect Dis* 24: 2107–2109. doi: 10.3201/eid2411.170264.
- Lu Q, Yu J, Yu L, Zhang Y, Chen Y, Lin M, Fang X (2018) *Rickettsia japonica* infections in humans, Zhejiang Province, China, 2015. *Emerg Infect Dis* 24: 2077–2079. doi: 10.3201/eid2411.170044.
- Gao S, Li L, Zhou X, Dai X, Lu L, Chen Y, Han J, Huang X,

- Xiao Q, He H, Liu Q (2022) Fatal *Rickettsia japonica* infection complicating disseminated intravascular coagulation in Yichang, China. *Infect Drug Resist* 15: 6613–6623. doi: 10.2147/IDR.S383917.
11. Li W, Liu SN (2021) *Rickettsia japonica* infections in Huanggang, China, in 2021. *IDCases* 26: e01309. doi: 10.1016/j.idcr.2021.e01309.
  12. Qin XR, Han HJ, Han FJ, Zhao FM, Zhang ZT, Xue ZF, Ma DQ, Qi R, Zhao M, Wang LJ, Zhao L, Yu H, Liu JW, Yu XJ (2019) *Rickettsia japonica* and novel *Rickettsia* species in ticks, China. *Emerg Infect Dis* 25: 992–995. doi: 10.3201/eid2505.171745.
  13. Qi Y, Ai L, Zhu C, Ye F, Lv R, Wang J, Mao Y, Lu N, Tan W (2022) Wild hedgehogs and their parasitic ticks coinfecting with multiple tick-borne pathogens in Jiangsu Province, Eastern China. *Microbiol Spectr* 10: e0213822. doi: 10.1128/spectrum.02138-22.
  14. Araki M, Takatsuka K, Kawamura J, Kanno Y (2002) Japanese spotted fever involving central nervous system: two case reports and a literature review. *J Clin Microbiol* 40: 3874–3876. doi: 10.1128/JCM.40.10.3874-3876.2002.
  15. Kodama K, Noguchi T, Chikahira Y (2000) Japanese spotted fever complicated by acute respiratory failure. *Kansenshogaku Zasshi* 74: 162–165. doi: 10.11150/kansenshogakuzasshi1970.74.162.
  16. Fujita S, Akagi T, Yamamoto T, Morita Y (2019) Japanese spotted fever: a rare cause of arthritis. *Intern Med* 58: 2117–2118. doi: 10.2169/internalmedicine.2600-19.
  17. Kodama K, Senba T, Yamauchi H, Chikahira Y, Fujita H (2001) Japanese spotted fever associated with multiorgan failure. *J Infect Chemother* 7: 247–250. doi: 10.1007/s101560170021.
  18. Himeji D, Nasu S, Tanaka GI, Masuda K, Matsuura R (2021) Japanese spotted fever complicated with life-threatening ventricular tachycardia and acute cardiac damage. *Cureus* 13: e18408. doi: 10.7759/cureus.18408.
  19. Wada K, Sakaeda H, Aono R, Chiya S (2008) Fulminant Japanese spotted fever - the second fatal case in Japan. *Kansenshogaku Zasshi* 82: 77–81. doi: 10.11150/kansenshogakuzasshi1970.82.77.
  20. Sando E, Suzuki M, Katoh S, Fujita H, Taira M, Yaegashi M, Ariyoshi K (2018) Distinguishing Japanese spotted fever and scrub typhus, central Japan, 2004-2015. *Emerg Infect Dis* 24: 1633–1641. doi: 10.3201/eid2409.171436.
  21. Noguchi M, Oshita S, Yamazoe N, Miyazaki M, Takemura YC (2018) Important clinical features of Japanese spotted fever. *Am J Trop Med Hyg* 99: 466–469. doi: 10.4269/ajtmh.17-0576.
  22. Tai K, Iwasaki H, Ikegaya S, Takada N, Tamaki Y, Tabara K, Ueda T (2014) Significantly higher cytokine and chemokine levels in patients with Japanese spotted fever than in those with Tsutsugamushi disease. *J Clin Microbiol* 52: 1938–1946. doi: 10.1128/JCM.03238-13.
  23. Sakabe S, Tanaka H, Nakanishi Y, Toyoshima H (2022) The clinical course of 239 cases of Japanese spotted fever in Ise Red Cross Hospital, 2006-2019. *J Infect Chemother* 28: 211–216. doi: 10.1016/j.jiac.2021.10.014.
  24. Sahni A, Fang R, Sahni SK, Walker DH (2019) Pathogenesis of rickettsial diseases: pathogenic and immune mechanisms of an endotheliotropic infection. *Annu Rev Pathol* 14: 127–152. doi: 10.1146/annurev-pathmechdis-012418-012800.
  25. Walker DH, Ismail N (2008) Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nat Rev Microbiol* 6: 375–386. doi: 10.1038/nrmicro1866.
  26. Bechelli J, Smalley C, Zhao X, Judy B, Valdes P, Walker DH, Fang R (2016) MyD88 mediates instructive signaling in dendritic cells and protective inflammatory response during rickettsial infection. *Infect Immun* 84: 883–893. doi: 10.1128/IAI.01361-15.
  27. Bechelli J, Smalley C, Milhano N, Walker DH, Fang R (2015) *Rickettsia massiliae* and *Rickettsia conorii* Israeli spotted fever strain differentially regulate endothelial cell responses. *PLoS One* 10: e0138830. doi: 10.1371/journal.pone.0138830.
  28. Matsuura H (2021) Japanese spotted fever and rickettsial pneumonia. *Qjm* 114: 261–262. doi: 10.1093/qjmed/hcaa331.
  29. Guccione C, Rubino R, Colomba C, Anastasia A, Caputo V, Iaria C, Cascio A (2022) Rickettsiosis with pleural effusion: a systematic review with a focus on rickettsiosis in Italy. *Trop Med Infect Dis* 7: 11. doi: 10.3390/tropicalmed7010011.
  30. Stewart AG, Stewart AGA (2021) An update on the laboratory diagnosis of *Rickettsia* spp. infection. *Pathogens* 10: 1318. doi: 10.3390/pathogens10101319.
  31. Dhawan S, Robinson MT, Stenos J, Graves SR, Wangrangsimakul T, Newton PN, Day NPJ, Blacksell SD (2020) Selection of diagnostic cutoffs for murine typhus IgM and IgG immunofluorescence assay: a systematic review. *Am J Trop Med Hyg* 103: 55–63. doi: 10.4269/ajtmh.19-0818.
  32. Robinson MT, Satjanadumrong J, Hughes T, Stenos J, Blacksell SD (2019) Diagnosis of spotted fever group rickettsia infections: the Asian perspective. *Epidemiol Infect* 147: e286. doi: 10.1017/S0950268819001390.
  33. Abdad MY, Abou Abdallah R, Fournier PE, Stenos J, Vasoo S (2018) A concise review of the epidemiology and diagnostics of rickettsioses: *Rickettsia* and *Orientia* spp. *J Clin Microbiol* 56: e01728–17. doi: 10.1128/JCM.01728-17.
  34. Chiu CY (2013) Viral pathogen discovery. *Curr Opin Microbiol* 16: 468–478. doi: 10.1016/j.mib.2013.05.001.
- Gu W, Miller S, Chiu CY (2019) Clinical metagenomic next-generation sequencing for pathogen detection. *Annu Rev Pathol* 14: 319–338. doi: 10.1146/annurev-pathmechdis-012418-012751.

### Corresponding author

Xiaowei Xu, PhD.

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, 79 Qingchun Road, Hangzhou, Zhejiang Province, P. R. China, 310003  
Tel: +86 13605708066  
Email: xxw69@zju.edu.cn

**Conflict of interests:** No conflict of interests is declared.