



Short Communication

Diagnosing and reintegrating traceability of infectious diseases via metagenomic next-generation sequencing: Study of a severe case of *Rickettsia japonica* infection



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ABSTRACT

Background: In this study, we present a case of Japanese spotted fever (JSF) caused by *Rickettsia japonica* and use this case to investigate the process of diagnosing and reintegrating traceability of infectious diseases via metagenomic next-generation sequencing (mNGS).

Methods: From data relating to epidemiological history, clinical and laboratory examinations, and mNGS sequencing, a diagnosis of severe JSF was concluded.

Results: A detailed field epidemiological investigation discovered parasitic *Haemaphysalis longicornis* from a host animal (dog) in the domicile of the patient, within which *R. japonica* was detected, along with a diverse array of other potentially pathogenic microorganisms that could cause other infectious diseases.

Conclusion: The mNGS provided an efficient method to diagnose JSF infection. This methodology could also be applied to field epidemiological investigations to establish the traceability of infectious diseases.

1. Introduction

Japanese spotted fever (JSF) is a tick-borne rickettsiosis caused by *Rickettsia japonica*. Initially documented in 1984 in Japan [1], subsequent cases have been identified in several countries including the Philippines [2], South Korea [3], and Thailand [4]. The typical clinical presentation includes a fever, rash, and tick bite eschars, constituting the classical triad. Additionally, liver dysfunction, headache, and disseminated intravascular coagulation syndrome have been reported as associated symptoms [5]. In various provinces of China, including Anhui, Henan, Anhui, Hubei, and Zhejiang, cases of JSF have been documented [6–10], with a higher number of reports in central and eastern China. This study provides details on a specific case of JSF caused by *R. japonica* in Shaanxi,

northwest China, as well as supporting evidence for this infection from field epidemiological investigations.

2. Materials and methods

2.1. Case and data collection

A male patient presented with a fever (39°C on May 29, 2021), rash, and oliguria in Shanyang County, Shaanxi Province, China in June, 2021. Data relating to clinical and laboratory examinations and epidemiological history during different medical stages were recorded and analyzed. Peripheral blood samples taken from different sites were also collected for blood culture and metagenomic next-generation sequencing (mNGS).

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2.2. Metagenomic sequencing and data analysis

The mNGS detection of peripheral blood samples was performed by Jinyu Clinical Laboratory Center, Zhengzhou. Nucleic acid extraction of tick samples was performed using the Ex-DNA/RNA nucleic acid extraction kit (TIAN LONG, Xi'an, China), according to the manufacturer's instructions. Sequence libraries were pooled and generated using the Next Ultra DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) and sequenced on an Illumina Hiseq platform. The filtered sequence reads were *de novo* assembled using Megahit v1.0.3 [11] with default parameters. The taxonomic classification was performed using kraken2 [12] and relative abundance was computed using bracken [13]. Then, the results were visualized using the Pavian program (<https://fbreitwieser.shinyapps.io/pavian/>). The metagenomic assembled genomes were generated by semibin [14] and the output was based on >50% completeness and <10% contamination. Diamond [15] was used to determine protein identity and virulence factor matches in the virulence factor database (VFDB) [16].

3. Results

3.1. Case report

On June 7, 2021, a male patient aged 59, from Shanyang County, Shaanxi Province, China, was admitted to Tangdu Hospital, Xi'an, presenting with symptoms including a fever, rash, and oliguria. Prior to this, the patient had received antibiotic treatment at a local county hospital since June 2. Although the patient's body temperature normalized, intermittent fever and rash persisted. On June 5, the patient began experiencing symptoms of type I respiratory failure, fever, rash, oliguria, unidentified shortness of breath, and a rapid decline in platelet count. As a result, he was transferred to Tangdu Hospital on June 7. The patient reported working in woodland and fields before the onset of the disease. At admission, the patient had a black eschar on the back of his left foot and his left calf, surrounded by desquamation (Fig. 1A), with a scattered rash across his whole body but especially on his limbs. The rash did not fade when pressed. An abdominal ultrasound showed diffuse lesions on both kidneys with peritoneal effusion. The results of laboratory tests are presented in Table S1. Blood gas analysis revealed a pH value of 7.38, PaO₂ of 69.9 mmHg, PaCO₂ of 30.1 mmHg, an oxygenation index of 333 mmHg, and a lactic acid level of 5.0 mmol/L. Chest X-ray examination at the bedside showed inflammation of both lungs. After admission, a sodium lactate Ringer's injection (1000 mL) was administered intravenously to correct hypotension, but the patient quickly developed restlessness, vague consciousness, skin spots all over the body, cold limbs,

a drop in blood pressure to 80/50 mmHg, and oxygen saturation in peripheral blood fluctuated between 89%–93% with mask oxygen inhalation set at 8 L/min, indicating that the patient was experiencing hypotension shock and acute respiratory distress syndrome. Pressure support ventilation, synchronous gap mandatory ventilation, and mechanical ventilation with ventilator support were applied. The patient was treated with dexmedetomidine (0.04 mg/h) combined with midazolam (5 mg/h) sedation, norepinephrine (0.6 mg/h) pressor, dexamethasone (10 mg) anti-inflammation, a plasma 400 mL intravenous drip, and a platelet 10 IU intravenous drip. After 2 h of rescue, the patient was in a state of sedation, the skin spots disappeared, and peripheral blood oxygen saturation increased to 100%. On June 8, the patient's body temperature rose to 40.1°C, accompanied by shivering and cervical ankylosis. Controlled blanket cooling, doxycycline (0.1 g every 12 h) gastric tube infusion, and a meropenem (1 g every 8 h) intravenous drip were applied. During the fever, two peripheral blood samples from different sites were collected for blood culture on June 8. The results of a β -D-glucan test and the Weil–Felix reaction were negative. The peripheral blood samples were also submitted for mNGS, which confirmed the presence of *R. japonica* (Fig. 1B). Considering the epidemiological history, the results of clinical and laboratory examinations, and the mNGS data, JSF was diagnosed.

The treatment regimen was adjusted as follows: doxycycline (0.1 g every 12 h) gastric tube injection plus a moxifloxacin (0.4 g once a day) intravenous drip, and mannitol dehydration to reduce intracranial pressure, and plasma, human serum albumin, platelet and liver protection therapy. The patient exhibited a gradual recovery with body temperature stabilizing, increased clarity of consciousness, an improved oxygenation index, and an increase in 24-h urine volume to 1000–2000 mL. The rash on the trunk and legs disappeared, while the rash on both feet persisted. Additionally, the platelet count, coagulation function, renal function, and procalcitonin levels returned to normal, and there was a significant decrease in ALT and AST levels. Following this continued treatment regimen, the patient achieved a successful recovery and was discharged after several days.

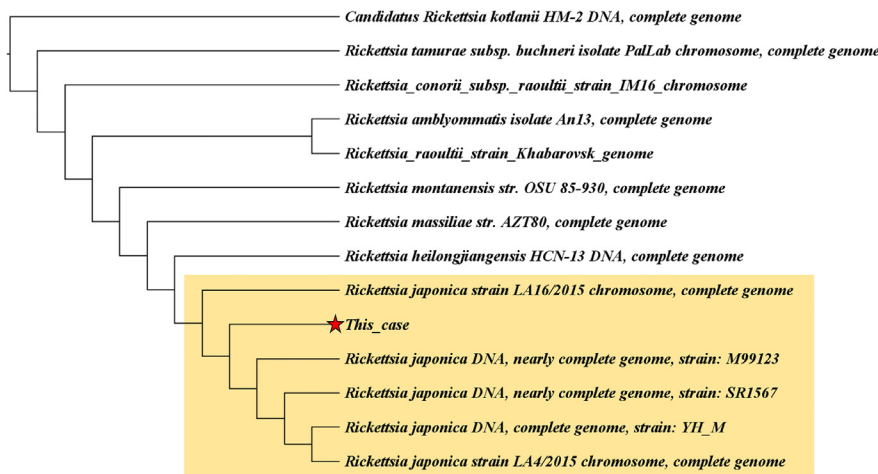
3.2. Field epidemiological investigation

Subsequently, a more detailed epidemiological investigation was conducted by a well-trained epidemiological investigator while the patient was conscious, particularly focusing on the time of onset of illness, the place of residence and sojourn, the history of contact with others, and exposure to insect bites. Furthermore, a field epidemiological investigation was conducted following the exchange of information between clinicians and epidemiologists with the assistance of the local Center for

A



B



C

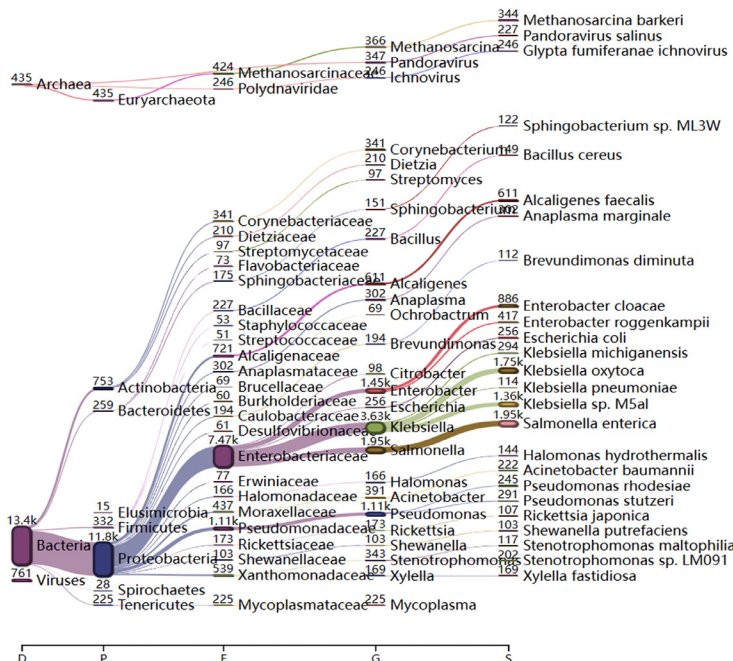


Fig. 1. Clinical manifestations mNGS results of the case and metagenomic microbial classification results of parasitic ticks. (A) Eschar and erythematous rashes scattered across the legs (b, c) and feet (a) of the patient. (B) Neighbor-joining phylogenetic tree of the sequences, obtained by metagenomic next-generation sequencing, with high identity based on the basic local alignment search tool (BLAST) algorithm. Red stars indicate sequences from this case. (C) Sankey plot of the metagenomic microbial classification results in *Haemaphysalis longicornis* collected from the host animal (dog). The contigs are classified as D, domain; P, phylum; F, family; G, genus; S, species, from the right to left column. The segments are colored according to taxonomy level. The numbers on the nodes represent the count of contigs classified in the metagenome data.

Disease Control and Prevention (CDC), prompted by the need to trace infectious diseases and address public health concerns. We collected 24 *Haemaphysalis longicornis* from a host animal (dog) in the domicile of the patient and tested these ticks for pathogenic microorganisms through mNGS. The results of metagenomic analysis revealed a diverse array of potential pathogenic microorganisms including *Anaplasma marginale* and *Mycoplasma* in the *H. longicornis* samples, in addition to *R. japonica* (Fig. 1C).

4. Discussion

The classified diagnosis of rickettsial infection depends on serological examination. In this case report, the results of the Weil–Felix reaction for the patient during the acute phase of disease were negative, thus a diagnosis could not be classified based on serological examination. It is now possible to extract nucleic acid sequences directly from clinical specimens using mNGS, which helps in the rapid and accurate diagnosis of difficult-to-diagnose infections and critically ill patients. Although the number of *Rickettsia* in peripheral blood according to mNGS in this case was low, the test results may have been influenced by the anti-infection treatment administered to the patient at the local hospital. Hence, a thorough assessment encompassing the patient's epidemiological history, clinical symptoms and signs, routine laboratory examination, and mNGS auxiliary examination was conducted, leading to the diagnosis of JSF. Considering the patient's concurrent hypotension shock, liver and kidney function injury, consciousness disorder, acute respiratory distress syndrome, and multiple organ failure, this case was ultimately classified as severe.

Field epidemiology investigation often serves as guidance for selecting and implementing early interventions to prevent infectious diseases from developing [17]. In this case study, we identified another three suspected cases with black eschars or a whole body rash and fever without an obvious cause from April to June, 2021, in the local hospital of Shanyang county during further investigations. A fever and cough were reported by all three patients. Other nonspecific symptoms included asthenia (2/3) and rash (2/3). Because the three suspected cases had been cured before our identification, we were not able to confirm the presence of the pathogen in their blood. However, we were able to collect parasitic ticks from the resident dog, but not from the woodland or fields under investigation. Our findings indicated that this region was a potential epidemic focus and that small invertebrates could potentially serve as sentinels of tick-borne infectious diseases. This was supported by subsequent metagenomic analysis of parasitic ticks from the dog in this case. Common tick-borne pathogens such as *C. burnetii*, *A. marginale*, and *Mycoplasma* were also identified through taxonomy profiling of mNGS reads, which means

that there is still a risk of human infection with tick-borne diseases through co-infection in this region. Binning analysis was performed from the raw sequencing data of all samples and only two metagenomic assembled genomes were classified as belonging to the *Coxiella* genus (bin183) and *Francisella* sp002095075 (bin73) with potential virulence factors (Table S2). It is therefore vital that the CDC urgently enhance prevention and control measures to protect against the local risk of infection. In addition, because metagenomic sequencing of tick samples was performed by pooling multiple samples for the purpose of pathogen screening, the occurrence of pathogen coinfection and pathogen–pathogen interactions could not be explored in this study. This will be the subject of our future studies.

Overall, in abnormal infectious disease cases, effective communication among clinicians, epidemiologists, and CDCs, is crucial to improve collaboration among different departments and recognize public health threats as early as possible. Furthermore, through successful collaboration, interventions that reduce or prevent illness or death could be selected and implemented more effectively.

5. Conclusion

In this study, we present a severe case of JSF infected by *R. japonica*. Our findings demonstrate that mNGS, in combination with clinical information and field epidemiological investigations, is a viable approach for establishing the traceability of infectious diseases.

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Authors contributions

ZL, JH, and YW: Data curation, writing, and original draft preparation. ZS, KL, and YW: Conception and design of the manuscript and the acquisition of data. ZS and KL: Critical revision of the manuscript for important intellectual content and final approval of the version to be submitted. ZS, KL, ZL, and JH: Field epidemiological investigation and sample collection. SS, ZH, and JH: Performed the experiments. ZL: Data analysis and visualization. All authors read, commented on, and approved the final manuscript.

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Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

Ethical approval not required.

Informed consent

Consent for publication of the case was granted by the patient.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.imj.2024.100094](https://doi.org/10.1016/j.imj.2024.100094).

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