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Case report

The presence of foci of *Rickettsia conorii* infection in China

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ABSTRACT

China is not considered as an endemic area of *Rickettsia conorii*, so there is no routine clinical way to diagnose this infection. This study aims to determine whether 2 febrile patients who had a tick bite in East China were indeed infected with *R. conorii.* The citrate synthase gene (*gltA*) was amplified with universal rickettsial primers by real-time fluorescent PCR from the patients' blood samples. Nested PCR was used to amplify the outer membrane protein A gene (*ompA*) for positive specimens. PCR products were further identified and analyzed through nucleic acid sequencing. Positive amplification of the *gltA* and *ompA* genes was found in both patients. The nucleotide sequences (303 bp) of the *ompA* gene of the 2 patients had high homology (99%) with the *R. conorii* Indian tick typhus strain in GenBank. A more than 4-fold increase in IgG against *R. conorii* provided supportive evidence of SFG Rickettsia infection. And the rapid recovery after doxycycline treatment also supported a rickettsial cause for the disease. Physicians in East China should be aware of human infections with *R. conorii.* PCR-based diagnostic methods offer a rapid and precise way to diagnose rickettsiosis, improving patient identification and management.

1. Introduction

Rickettsia conorii (*R. conorii*) belongs to the spotted fever group rickettsiae, which currently includes 4 subspecies [1–3]. *R. conorii* infection can cause fever, rash, and flu-like symptoms. China is not considered endemic, so there is no routine clinical way to diagnose the disease. The first case of *R. conorii* infection identified by next-generation sequencing was recently reported in China [4]. In 2020, there were 2 acute febrile patients with a history of tick bites, in whom rickettsiosis was considered. To clarify the etiology, we synthesized universal rickettsial primers and used them for molecular biological identification of the suspected cases.

2. Case presentation

In the summer of 2020, we admitted 2 patients (Patient 1 and Patient 2) with acute fever of unknown origin from Taian City and Zibo City in Shandong Province, China. Patient 1 was a 72-year-old female, and patient 2 was a 62-year-old male. The patients were both elderly farmers who lived in hilly areas. Both patients described a history of tick bites 1 week before disease onset. The main clinical symptoms were fever, headache, fatigue, loss of appetite, nausea, and other flu-like symptoms. Patient 2 developed rashes on the third day of fever. At admission, both patients had leukopenia, thrombocytopenia, and elevated hepatic aminotransferase levels. Routine bacterial cultures of the blood samples collected from the patients on admission were negative, as were the viral nucleic acid tests of the throat swabs. On physical examination after admission, no eschars or ulcers were found in either patient. Scattered maculopapular rashes were seen on the limbs and trunk in patient 2 (Fig. 1). The possibility of infection with atypical pathogens was considered, but both patients had negative Weil–Felix test results and negative serological test results for *Coxiella burnetii, Rickettsia*

Abbreviations: IFA, indirect immunofluorescence assay; gltA, citrate synthase gene; ompA, outer membrane protein A gene; SFGR, spotted fever group rickettsiae.

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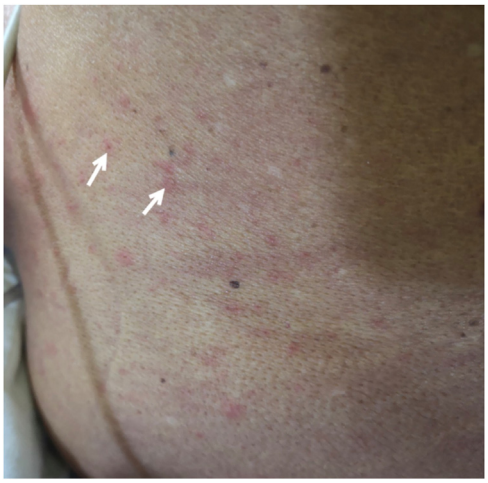

Fig. 1. Rash on the back of patient 2. White arrow indicates scattered maculopapular rashes.

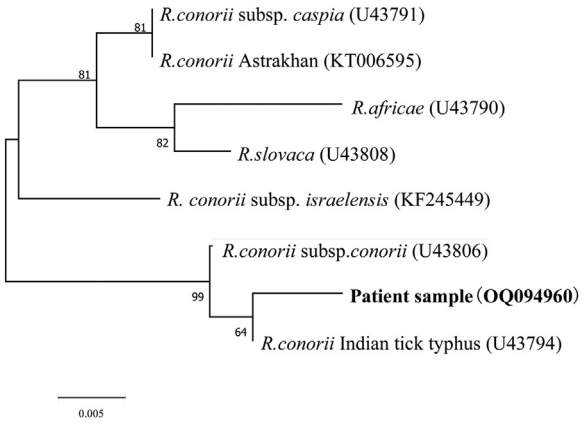

Fig. 2. Phylogenetic tree of *Rickettsia conorii* based on the outer membrane protein A gene. Sequences of representative viral strains were downloaded from National Center for Biotechnology Information public databases (https://www.ncbi.nlm.nih.gov) and aligned together using MEGA version 7.0 (https://www.megasoftware.net). A bootstrapping analysis of 1,000 replicates were conducted, and values >60 were considered significant and are shown. Scale bars indicate estimated evolutionary distance.

mooseri, and *Orientia tsutsugamushi*. Supplementary Table S1 lists the detailed epidemiological history and clinical and laboratory data. To clarify the underlying etiology, we performed PCR amplification of peripheral blood samples from the patients using universal rickettsial primers.

Total DNA was extracted from the peripheral blood samples of the patients using QIAamp DNA Blood Mini kits (Qiagen) according to the manufacturer's instructions. Nuclease-free water was used as a negative control for each sample in each PCR experiment analysis. TaqMan real-time quantitative PCR was used for preliminary detection of rickettsial DNA in samples. The citrate synthase gene (*gltA*) was selected as a target for amplification due to its specificity and conservation [5]. Supplementary Table S2 lists the details of the primers and probe nucleotide sequences used.

To complete the identification of *Rickettsia* species in DNA samples, the rickettsial outer membrane protein A gene (*ompA*) was amplified by nested PCR [6]. The nucleotide sequences of the primers are shown in Supplementary Table S2. The PCR products were electrophoresed in agarose gel, and the positively amplified DNA fragments were extracted and purified. The purified amplification products were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing. The nucleotide sequence reads obtained were aligned with the known *ompA* gene sequence in the NCBI database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using the neighbor-joining method of MEGA 7.0 software (the number of bootstrap replicates was set to 1000).

Real-time fluorescent PCR of the *gltA* gene indicated that the spotted fever group rickettsiae (SFGR) nucleic acids were positive in the peripheral blood samples of both patients in the acute phase. According to the comprehensive analysis of their epidemiological history (a recent history of tick bites), clinical manifestations, and laboratory test results, both patients had spotted fever. To specifically identify the *Rickettsia* species in the positive samples, the *ompA* gene fragments were amplified using nested PCR, yielding a 303-bp fragment, in both DNA samples (Supplementary Fig. S1) and were sequenced. The *ompA* gene sequences of different *Rickettsia* species were selected from GenBank and then compared with the obtained nucleotide sequences using BLAST software. The results revealed that the sequences of the 2 *ompA* gene fragments were 100% identical to each other. The *ompA* in these patients was 99% (302/303 bp) homologous to the corresponding sequences of *R. conorii* from *Rhipicephalus turanicus* from Xinjiang, China (GenBank accession nos. MF002512, KU364365, and KY069258), an *R. conorii* strain from *Apodemus agrarius* from Shandong, China (GenBank accession no. OM234679), and an *R. conorii* strain from India (GenBank accession no. U43794). Phylogenetic analysis based on the *ompA* gene (Fig. 2.) also supported that the pathogen was closely related to the *R. conorii* Indian tick typhus strain. We deposited the nucleotide sequence data for this pathogen into GenBank (accession no. OQ094960). The indirect immunofluorescence assay (IFA) (RCG-120, Fuller Laboratories) was employed to detect specific IgG antibodies against *R. conorii* in serum samples of the 2 patients. The IFA assay utilized the Moroccan strain of *R. conorii* as the substrate antigen. The serological data also confirmed the PCR and sequencing results. Paired serum samples from both patients showed a more than 4-fold increase in *R. conorii* titers. The patients' clinical symptoms and signs gradually disappeared after 2–3 days of oral doxycycline treatment. After 15 days of treatment, both patients had recovered and were discharged from the hospital.

To further clarify the prevalence of *R. conorii*, we used the same method to detect *R. conorii* antibodies in

the peripheral blood samples of 41 patients with fever and thrombocytopenia syndrome collected from 2018 to 2019 for *R. conorii* antibodies. These blood samples had a seropositive rate for *R. conorii* of 5/41 (12%).

3. Discussion

R. conorii is an obligate intracellular Gram-negative SFGR bacterium. *Rhipicephalus sanguineus* is its main vector and reservoir host [7]. Some strains closely related to *R. conorii* were later discovered, including Indian tick typhus rickettsia (ITTR) [1], Israel spotted fever rickettsia (ISFR) [2], and Astrakhan fever rickettsia (AFR) [3]. Genetic analysis found that these strains were similar in gene sequence but differed in endemic regions and clinical manifestations, and together they constituted the *R. conorii* complex [8], which is currently known to be distributed in areas along the Mediterranean coast, Africa, southern Russia, the Middle East, India, and Pakistan [7].

Our findings suggest that acute febrile tick-borne disease caused by *R. conorii* is prevalent in Shandong Province. The gene fragments of *R.* conorii were initially detected in ticks collected during 2013–2014 in Xinjiang, western China [9]. Additionally, *R. conorii* DNA has been identified in the spleens of rodents collected between 2013 and 2015, suggesting that these rodents may serve as reservoir hosts for *R. conorii* in Qingdao, Shandong [10]. In addition to our report of the first human infection with *R. conorii* in 2019 [4], this is currently the second report of human infection with *R. conorii* in Shandong Province. Second, a serological review conducted among individuals with a clear history of contact with ticks showed a certain positive rate of *R. conorii* antibodies. These findings indicate that people in this area may have been exposed to *R. conorii* at some point in the past. In addition, the diversity of ticks increases the chances of pathogen transmission in Shandong [11]. These results suggest the presence of natural foci of Mediterranean spotted fever in Shandong Province. *R. conorii* infection may be more common in Shandong than is currently known, and there may be occult infections.

These results indicate that there are basic prerequisites for *R. conorii* to infect humans in China. As the understanding of *Rickettsia* improves and molecular diagnostic technologies advance, more cases of human rickettsial infection and new *Rickettsia* species may be discovered. Before this study, at least 6 SFGR genotypes that cause human infection had been identified in China [12–17]. Climate change as well as various biological factors has contributed to an increase in tick-borne diseases [11]. The outbreak of the COVID-19 pandemic at the end of 2019 suggested that it is necessary to further strengthen the prevention and control system for emerging infectious diseases and improve pathogen discovery, identification, and clinical diagnosis. In follow-up studies, we will conduct a more comprehensive investigation into the epidemiologic and genomic characteristics of *R. conorii* to gain a clearer understanding of its transmission vector and epidemiological trends in China.

The study has several limitations. First, we only sequenced the *ompA* gene for the identification of a new Rickettsia species. Additionally, due to the low concentration of rickettsia and incomplete sequencing in the blood sample in the 2019 case, direct comparisons with the samples obtained in this study were not possible. We were unable to conduct a comprehensive analysis of the genetic differences and evolutionary relationships between species.

When patients present with similar clinical manifestations, as the 2 patients in this study did, it is crucial to consider the possibility of rickettsial infection. PCR-based laboratory diagnostic methods offer a rapid and precise method of diagnosing rickettsiosis, enabling better identification and management of patients suffering from these infections.

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Author contributions

W.G.: Conceptualization, resources, writing – review & editing; X.N.N.: Methodology, investigation, writing – original draft; L.H.: Project administration, writing – original draft; Z.W.L.: Visualization, writing – original draft; Q.C.M.: Investigation; W.S.: Data curation, investigation; C.C.Y.: Supervision

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Declaration of competing interest

W.G. is an editorial board member for the Infectious Medicine and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

Data available statement

All data generated or analyzed during this study are included in this published article. The nucleic acid sequences obtained in this study were submitted to the NCBI database (GenBank accession no. OQ094960).

Ethics statement

This study was approved by Shandong University Qilu Hospital human research protection committee (IRB # KYLL-2019-268). All patients signed consent forms.

Informed consent

Both patients provided written informed consent at the time of entering this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imj.2023.09.002.

References

[1] D. Raoult, V. Roux, Rickettsioses as paradigms of new or emerging infectious diseases, Clin. Microbiol. Rev. 10 (4) (1997) 694–719, doi:10.1128/CMR.10.4.694.
[2] B.J. Cwikel, J. Ighbarieh, I. Sarov, Antigenic polypeptides of Israeli spotted fever isolates compared with other spotted fever group rickettsiae, Ann. N. Y. Acad. Sci. 590 (1990) 381–388 tb42244.x, doi:10.1111/j.1749-6632.1990.
[3] M. Drancourt, L. Beati, I. Tarasevich, et al., Astrakhan fever *Rickettsia* is identical to Israel tick typhus *Rickettsia*, a genotype of the *Rickettsia conorii* complex, J. Infect. Dis. 165 (6) (1992) 1167–1168, doi:10.1093/infdis/165.6.1167.
[4] N. Xu, W. Gai, Y. Zhang, et al., Confirmation of *Rickettsia conorii* subspecies indica infection by next-generation sequencing, Shandong, China, Emerg. Infect. Dis. 27 (10) (2021) 2691–2694, doi:10.3201/eid2710.204764.
[5] J. Stenos, S.R. Graves, N.B. Unsworth, A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae, Am. J. Trop. Med. Hyg. 73 (6) (2005) 1083–1085.
[6] N. Jia, Y.C. Zheng, J.F. Jiang, et al., Human infection with candidatus *Rickettsia tarasevichiae*, N. Engl. J. Med. 369 (12) (2013) 1178–1180, doi:10.1056/NEJMc1303004.
[7] C. Rovery, D. Raoult, Mediterranean spotted fever, Infect. Dis. Clin. North Am. 22 (3) (2008) 515–530 ix, doi:10.1016/j.idc.2008.03.003.
[8] Y. Zhu, P.E. Fournier, M. Eremeeva, et al., Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*, BMC Microbiol 5 (2005) 11, doi:10.1186/1471-2180-5-11.
[9] L.P. Guo, S.H. Jiang, D. Liu, et al., Emerging spotted fever group rickettsiae in ticks, northwestern China, Ticks Tick Borne Dis. 7 (6) (2016) 1146–1150, doi:10.1016/j.ttbdis.2016.08.006.
[10] X.L. Gu, R. Wang, C.M. Zhou, et al., Natural Mediterranean spotted fever foci, Qingdao, China, Emerg. Infect. Dis. 28 (12) (2022) 2524–2527, doi:10.3201/eid2812.221097.
[11] G.P. Zhao, Y.X. Wang, Z.W. Fan, et al., Mapping ticks and tick-borne pathogens in China, Nat. Commun. 12 (1) (2021) 1075, doi:10.1038/s41467-021-21375-1.
[12] N. Jia, Y.C. Zheng, L. Ma, et al., Human infections with *Rickettsia raoultii*, China, Emerg. Infect. Dis. 20 (5) (2014) 866–868, doi:10.3201/eid2005.130995.
[13] H. Li, X.M. Cui, N. Cui, et al., Human infection with novel spotted fever group *Rickettsia* genotype, China, 2015, Emerg. Infect. Dis. 22 (12) (2016) 2153–2156, doi:10.3201/eid2212.160962.
[14] Q. Lu, J. Yu, L. Yu, et al., *Rickettsia japonica* infections in humans, Zhejiang Province, China, 2015, Emerg. Infect. Dis. 24 (11) (2018) 2077–2079, doi:10.3201/eid2411.170044.
[15] N. Jia, J.F. Jiang, Q.B. Huo, et al., *Rickettsia sibirica* subspecies sibirica BJ-90 as a cause of human disease, N. Engl. J. Med. 369 (12) (2013) 1176–1178, doi:10.1056/NEJMc1303625.
[16] W. Yi, Study on aetiology of *Rickettsia heilongianggii* tick borne spotted fever, Chin. Public Health 7 (2001) 28–30.
[17] W. Liu, H. Li, Q.B. Lu, et al., Candidatus *Rickettsia tarasevichiae* infection in eastern central China: a case series, Ann. Intern. Med. 164 (10) (2016) 641–648, doi:10.7326/M15-2572.