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Case Report

Q fever diagnosed using metagenomic next-generation sequencing in Guangdong Province, China

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ABSTRACT

Q fever is a zoonotic disease caused by infection with *Coxiella burnetii* (*C. burnetii*). Due to its atypical symptoms and the absence of specific detection methods, Q fever is underdiagnosed commonly. Herein, we report a case of Q fever confirmed by metagenomic next-generation sequencing (mNGS) in March 2024 in Guangdong Province, China. The patient initially experienced fever and was admitted to hospital six days later. Despite a series of laboratory tests conducted at the hospital, the pathogen remained undetermined. Ten days after admission, mNGS revealed that the patient was infected with *C. burnetii*. The patient subsequently underwent treatment with doxycycline and recovered well. Epidemiological investigation revealed that the patient had been exposed to sheep infected with *C. burnetii* without any protective measures in Jiangxi Province, China. Based on the comprehensive results of mNGS, exposure history, clinical manifestations and treatment response, the patient was confirmed as a Q fever case. As a neglected and underestimated illness, Q fever necessitates an elevation in awareness among medical staff and the public. The public should be encouraged to take personal protective measures when exposed to livestock. Further research is needed to explore the rational application of mNGS in the diagnosis of uncommon and unknown diseases.

1. Introduction

Q fever is a zoonotic disease caused by infection with *Coxiella burnetii* (*C. burnetii*), which occurs worldwide. The largest outbreak occurred in the Netherlands between 2007 and 2010, where over 4,000 cases were reported [1]. China reported the first confirmed case of Q fever in 1950 and experienced several outbreaks during the 1960s in Inner Mongolia Autonomous Region, Sichuan Province, Yunnan Province, and Xizang Autonomous Region, affecting the public health and social economy in a certain [2]. For instance, Xizang Autonomous Region experienced an outbreak of Q fever in 1968, with a total of 46 cases recorded and one fatality resulting. A systematic review revealed that the overall prevalence of anti-*Coxiella* antibodies in people was 10 % through serological surveys in China [3]. Q fever is predominantly sporadic and maintained at a low level in Guangdong Province, China, with seroprevalence of 3.9 % [4]. Another study found that 5.8 % of patients with fever of unknown causes were diagnosed with Q fever using metagenomic next-generation sequencing (mNGS) between 2018 and 2019 in Zhuhai City, Guangdong Province [5]. Epidemiological and serological investigations have reported that most *C. burnetii* infections are associated with exposure to livestock and / or their products [2,6].

The symptoms of Q fever are atypical and resemble those of other respiratory infections, which can lead to misdiagnosis. Currently, Q fever has not been listed as a notifiable infectious disease in China. Therefore, infection with *C. burnetii* is usually not promptly considered

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in clinical evaluations. With the development of mNGS technology, more rare cases have been identified in recent years [7,8].

In March 2024, Guangdong Province reported a case of Q fever confirmed by mNGS. The patient was admitted to local hospital, all samples and clinical information were collected as part of routine clinical operations to make a clear diagnosis without any additional samples. This article does not include information that could potentially identify the patient described.

2. Case report

A 60-year-old male with a history of hypertension and fatty liver disease was diagnosed with Q fever on March 1, 2024, in Guangdong Province, China. The diagnosis was confirmed using mNGS after the patient had experienced repeated episodes of fever lasting for two weeks. The patient initially presented with a fever (reaching a temperature of 39 ℃) on February 16, 2024, and was diagnosed with "influenza-like illness" by a community clinic. On February 20, he sought further medical attention at a higher-level local hospital, and was admitted to the hospital with a diagnosis of acute enteritis on February 21 (Fig. 1).

During his hospitalization, the patient endured recurrent episodes of fever and chills, without coughing or expectoration. Examination revealed significant inflammation and hepatic damage, evidenced by abnormally heightened levels of C-reactive protein (CRP), aspartate aminotransferase (AST), and alanine aminotransferase (AST) (Fig. 2). He also developed a pulmonary infection, which was confirmed by a computed tomography scan on February 22.

During the initial 9 days of hospitalization, a series of tests were performed, including blood smear, Weil-Felix test, and cultures of both stool and blood. All these tests were negative for multiple pathogens, such as *typhoid*, *paratyphoid*, *typhus*, and *Vibrio cholerae*. Treatments with anti-inflammatory medications, either suprozon or meropenem, showed unsatisfactory outcomes. Since all suspected diagnoses based on clinical manifestations were ruled out, the hospital drew a blood sample from the patient on February 29 and dispatched it to a third-party medical laboratory for mNGS testing. Subsequently, on March 1, which was the tenth day of his admission, mNGS identified two sequence reads of *C. burnetii*. The *C. burnetii* genome contains 2,032,807 base pairs, and the total sequence length measured was 100 (bp), corresponding to a coverage rate of 0.0049 % (see Fig. S1). Nevertheless, the polymerase chain reaction (PCR) test for nucleic acids was negative in the aforementioned blood sample. Following the mNGS findings, the patient underwent treatment with doxycycline and recovered well, with cessation of fever after 2 days. Subsequently, the patient was discharged on March 6. Approximately three weeks post-discharge, a telephone follow-up was conducted, during which the patient reported being in good physical condition.

The patient resides in an urban area of Guangdong Province, China, and did not raise animals or birds. Between January 14 and February 15, 2024, he visited his older brother who lives in a rural area of Jiangxi Province, China. During that period, he assisted his older brother in slaughtering sheep on February 1, 2, 5, and 6, four occasions totally. According to the patient's account, the sheep were raised by his brother and there were no sick or deceased animals among them. Each slaughter involved two or three sheep and lasted for several hours. The patient's primary responsibilities during the process included catching the animals, shearing off their wool, and collecting innards using basins. Notably, he did not take any protective measures throughout the entire slaughter procedure.

The local Center for Disease Control and Prevention (CDC) promptly initiated an investigation. They collected two samples of sheep feces and two environmental samples of the ground from the sheepfold owned by the patient's older brother. The PCR results revealed that one sample of sheep feces and both environmental samples of the sheepfold ground tested positive for *C. burnetii* nucleic acid. Furthermore, the CDC identified three co-exposed individuals, but none of them reported experiencing any symptoms. In addition, ticks monitoring by the flagging method and rat density monitoring by trapping method were also carried out in the patient's community and surrounding parks. However, no ticks or rats were detected during these monitoring efforts.

3. Discussion

In this study, we identified a case of Q fever using mNGS in an individual who had been exposed to sheep approximately half a month before onset of illness. Positive nucleic acid tests in sample of sheep feces and environmental samples revealed that the exposed sheep were infected with *C. burnetii*, the pathogen responsible for causing Q fever.

This patient had participated in the slaughter of sheep four times, each session lasting for several hours, without wearing a mask or taking any other protective measures. The timeframe between the initial exposure and the onset of illness, as well as the final exposure and onset, ranged from 10 to 15 days, aligning with the typical incubation period for *C. burnetii* infection, which spans 7 to 40 days (with an average of 2 to 3 weeks) [9]. Additionally, surveillance conducted in the patient's urban community failed to detect any ticks or rats, which ruled out the possibility of infection in this area. The patient developed fever, pneumonia, and liver damage, consistent with the symptoms of Q fever [9]. And he recovered well after treatment with doxycycline, which is recognized as the most effective therapy for this disease [9,10]. Based on mNGS testing result, epidemiological and clinical investigations, we concluded that the patient was a Q fever case caused by infection with *C. burnetii* through exposure to infected sheep.

The most common route of acquiring an infection with *C. burnetii* is through the inhalation of bacteria produced by infected animals or their products, particularly sheep, goats, and cattle [10]. Prolonged or frequent contact with infected livestock, their placentas or wool can also lead to infection [11]. Moreover, ingesting unpasteurised milk presents another avenue for infection [12]. *C. burnetii* can be found in the urine, feces, vaginal mucus, parturient materials, and dairy products of infected animals [13]. We inferred that this patient was most likely infected through the inhalation of *C. burnetii* aerosols or contaminated dust originating from the excreta, viscera, placenta of infected sheep, or possibly through direct contact with infected sheep during the slaughtering process.

In China, cattle and sheep are the primary hosts of *C. burnetii*. Previous outbreaks have been associated with exposure to livestock and / or their products [2], the situation in our study was the same. Therefore, health authorities ought to enhance public awareness regarding Q fever, along with its preventive and control measures. They should remind individuals to adopt personal protective measures while engaging in breeding, slaughtering, and/or processing livestock products. Specifically, wearing masks and gloves is highly recommended. Furthermore, the government should strengthen the supervision over privately raised animals. In our study, the sheep raised privately by the patient's brother had never undergone testing for *C. burnetii*.

Q fever is a neglected infectious disease, despite being designated as a nationally notifiable condition in various countries, including the United States, 27 European Union / European Economic Area Countries and Australia [14]. Seroprevalence data for Q fever indicates higher actual exposure to *C. burnetti*, suggesting that Q fever is an important public health concern [15]. In China, Q fever has not been listed as a notifiable infectious disease, and it's under-reported and under-diagnosed [3]. The clinical manifestations of Q fever are atypical and present with influenza-like illness and pneumonia [9], which leads to a high misdiagnosis rate. Once it progresses to chronic Q fever, the patient may be at a higher risk for complications and mortality

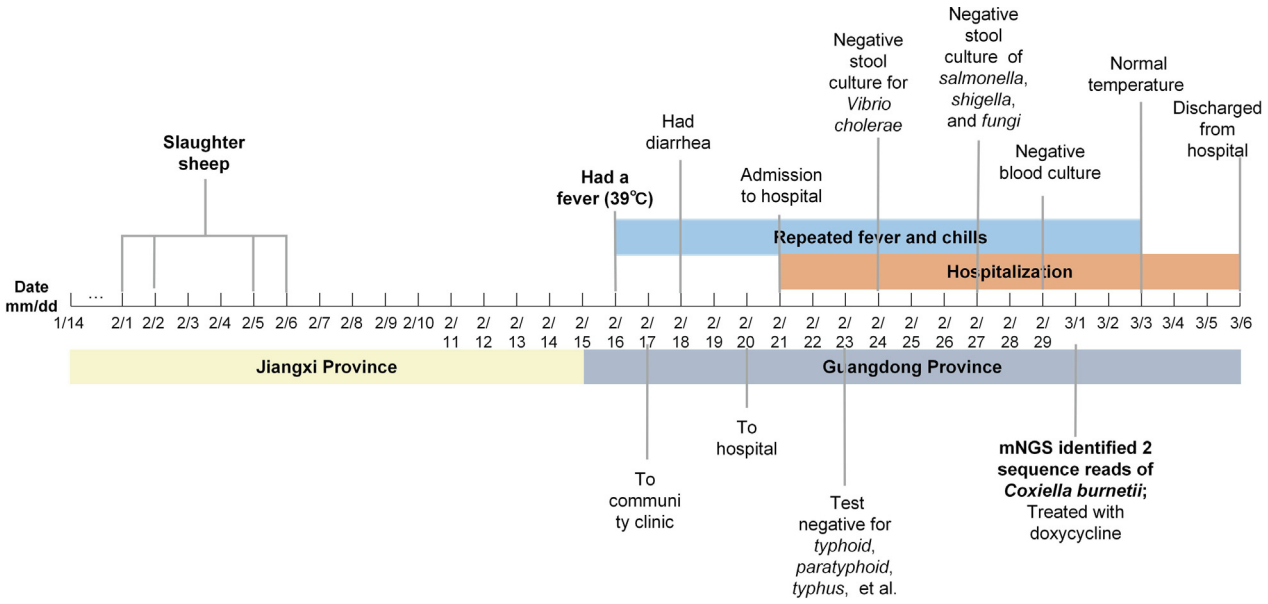

Fig. 1. The timeline of epidemiological and clinical investigation of the Q fever patient in Guangdong Province, China. Abbreviation: mNGS, metagenomic next-generation sequencing.

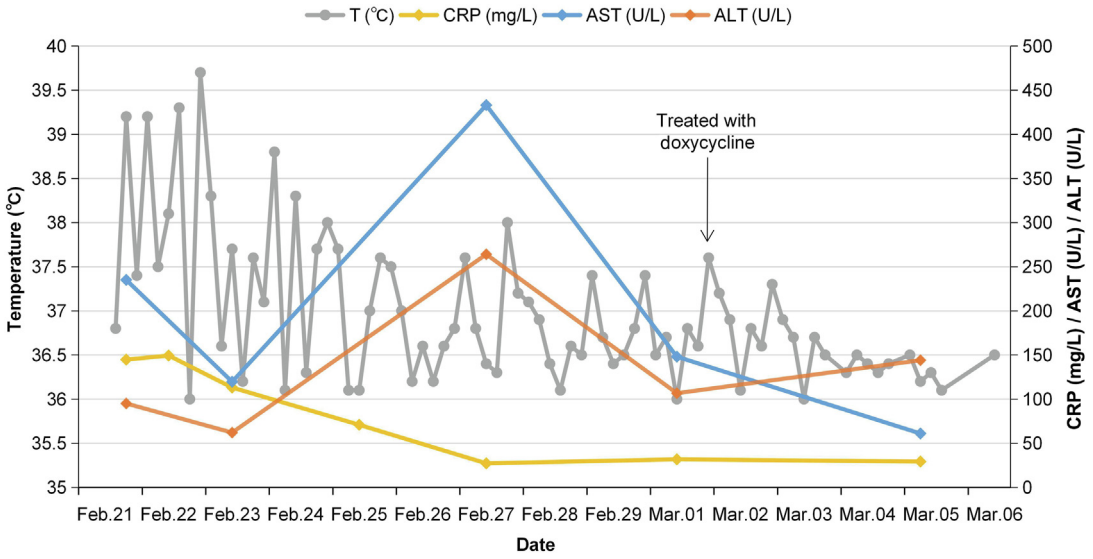

Fig. 2. The patient's temperature and laboratory test results during hospitalization. Abbreviations: CRP, C-reactive protein, normal range of 0–10 mol/L; AST, aspartate aminotransferase, normal range of 9–50 U/L; AST, alanine aminotransferase, normal range of 15–40 U/L.

[16], making early diagnosis crucial for timely and appropriate treatment. In our study, the clinicians did not suspect the patient's potential infection with C. burnetii until mNGS identification, indicating that Q fever is neglected in clinical practice. In the future, medical staff should enhance their awareness of Q fever. For patients with a suspected history of animal exposure, clinicians should consider the possibility of zoonotic diseases and carry out the necessary tests accordingly.

Prompt etiological diagnosis facilitates timely optimization of antimicrobial treatment, reducing the risk for complications, and promoting recovery. Generally, mNGS is unbiased with non-targeted identification and broad coverage, and is a potential tool for diagnosing unknown or uncommon diseases [17]. However, the implementation of mNGS faces several challenges, such as sequencing error rate, clinical data interpretation, and diagnostic heterogeneity among different laboratories [18]. Additionally, the high costs also limit its widespread application. Further research is needed to evaluate the sensitivity and reliability of mNGS, establish unified mNGS diagnostic standards or guidelines for its use, and assist early diagnosis of unknown and uncommon diseases. Moreover, the interpretation of mNGS results must be comprehensively integrated with clinical and epidemiological information.

The present study had some limitations. First, the nucleic acid PCR test for *C. burnetii* in the patient returned negative results, potentially due to prior anti-infective treatment or the natural disease progression, considering the approximately two-week interval between the onset of symptoms and blood sampling [10]. In addition, biosamples from the sheep raised by patient's brother were not collected as all the sheep had been killed. As such, the *C. burnetii* gene sequences between the patient and sheep could not be compared. Second, since Q fever is

an uncommon and neglected disease, neither the hospital nor the CDC had stockpiled detection reagents for anti-*C. burnetii* antibodies. Consequently, the mNGS results were not verified by serological testing. As a traditional diagnosis method, we suggest that at least larger hospitals and CDC laboratories should stock serological tests for *C. burnetii*.

In summary, based on the comprehensive results of mNGS, exposure history, clinical manifestations and treatment response, the patient described in this article was confirmed as a Q fever case caused by *C. burnetii* infection. Our study underscores the importance of raising awareness among medical staff and the public about Q fever to facilitate early diagnosis. We advocate for the rational use of traditional serological laboratory methods to confirm Q fever. With the development of mNGS technology, mNGS may be used as an auxiliary tool for identifying unknown and uncommon diseases in the future.

Ethics statement

The source of biological samples conforms to relevant regulations and ethical principles. The related content and purpose of the research are within the scope of standardized informed consent. After being reviewed by Medical Research Ethics Review Committee of Guangdong Provincial Center for Disease Control and Prevention, this study was determined to be exempt from ethical review.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Ting Hu: Writing – original draft, Methodology, Investigation. **Yuan Cheng:** Investigation. **Jia Wan:** Investigation. **Yandong Liu:** Investigation. **Yali Zhuang:** Writing – review & editing. **Mengxi Zhou:** Investigation. **Xin Zhang:** Methodology. **Xiaohua Tan:** Methodology. **Aiping Deng:** Methodology. **Meng Zhang:** Methodology. **Peng Wang:** Investigation. **Xiaoying Li:** Investigation. **Jun Zong:** Methodology, Investigation. **Lihong Cheng:** Methodology. **Min Kang:** Writing – review & editing, Funding acquisition.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2024.11.003.

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