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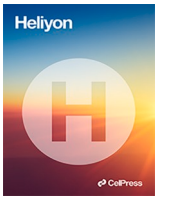

Research article

A case report of the metagenomic next-generation sequencing for timely diagnosis of a traveler with nonspecific febrile Q fever

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ABSTRACT

Q fever is a worldwide distribution disease caused by *Coxiella burnetii* (*C. burnetii*) , an obligate intracellular, Gram-negative acidophilic bacterium belonging to γ-proteobacterium. Most patients present with acute Q-fever accompanied by atypical flu-like symptoms, with only 1%–5% of cases may develop into persistent and focally infected foci, mainly manifest as endocarditis, osteomyelitis and prosthetic arthritis. In this case, the patient experienced an unexplained and uninterrupted fever up to 39.2 °C for a week, accompanied by chills and headaches, as well as abnormal liver function. The laboratory reported negative results for blood culture and respiratory-associated pathogens, however, the metagenomic next-generation sequencing (mNGS) reported that detection of 20 sequence reads of *C. burnetii* in the patient's peripheral blood. In addition, the patient had traveled to Sri Lanka, Iraq and Saudi Arabia before illness. In clinical, the treatment regimen was adjusted from empirically intravenous moxifloxacin 400 mg a day for 1 week to continuously oral minocyline 100 mg twice daily for 2 weeks. The patient was in good health without any adverse sequelae during outpatient visitation and the phone calls follow-up. In conclusion, the mNGS does provide an early and timely diagnostic basis for rare and difficult to culture pathogens, which contributes to the success of clinical anti-infection.

1. Introduction

Q fever is a globally distributed disease caused by *Coxiella burnetii*, an obligate intracellular, Gram-negative, acidophilic bacterium belonging to the γ-proteobacteria [1]. Since its first description in 1935 during an outbreak in Australia, knowledge of *C. burnetii* and its associated infections has dramatically increased. It has been defined as a potential bioterrorism pathogen by the U. S. Centers for Disease Control and Prevention, resulting in a public health threat of Q fever throughout the world [2]. Clinically, more than 60 % of

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cases present with flu-like symptoms during the acute phase [3]. In addition, there have been a few reports of *C. burnetii*-related pneumonia, acute hepatitis, acute cholecystitis, cardiac involvement (including pericarditis, myocarditis, and acute endocarditis), neurophlegmon, lymphadenitis, and autoimmunity [1]. However, only 1–5% of cases develop persistent focal infections, including endocarditis, osteomyelitis, prosthetic arthritis, chronic hepatitis, and neurologic lesions [3]. Therefore, rapid diagnosis of Q fever and timely and effective treatment are key to preventing progression.

For decades, the cultivation of strictly intracellular bacteria such as *C. burnetii* has been a challenge in conventional laboratories. Methods include culturing in embryonated eggs, animal inoculation, co-culture with amoebae and growth in the axenic medium developed by the NIH(National Institutes of Health) in 2009 [4], and all are time-consuming and demand specific technology and a biosafety level 3 laboratory. In addition, serological methods and/or polymerase chain reaction (PCR) technology used for the diagnosis of Q fever are not routinely performed in non-epidemic areas, leading to failures in diagnosing Q fever.

Herein, we present a 53-year-old man with uninterrupted fever for one week. *C. burnetii* DNA was detected in the peripheral blood using metagenomic next-generation sequencing (mNGS), which proved to be a crucial technology for detecting this rare pathogen.

2. Methods

This study was approved by the research ethics board at Peking University People's Hospital (ID: 2023PHB149-001).

2.1. Laboratory and imaging examinations

Laboratory evaluation for infectious etiologies was extensive and included tuberculosis testing by interferon-γ release assay; serologic testing for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumoniae*; PCR testing for influenza A and B viruses, *M. pneumoniae*, *C. pneumoniae*, respiratory syncytial virus, adenovirus, parainfluenza virus, Coxsackie virus, and SARS-CoV-2; and aerobic and anaerobic cultures of peripheral blood. Non-infectious causes of fever were investigated using autoantibody titers and tumor markers. The patient also underwent thyroid ultrasonography, brain magnetic resonance imaging (MRI), and chest, abdominal, and pelvic enhanced computed tomography (CT).

2.2. mNGS

The patient's blood was sent for pathogen detection using mNGS (Nextseq550; Illumina, San Diego, CA, USA) on the day of hospitalization. The procedure was as follows: the PathoXtract® WYXM03010S microbial cell-free deoxyribonucleic acid (mcfDNA) enrichment extraction kit (WillingMed, Beijing, China) was used to extract mcfDNA from 500 μL supernatant obtained after centrifugation of the patient's peripheral blood at 1900 g and 4 °C for 10 min. Following the manufacturer's instructions, the operators used the KAPA DNA HyperPrep Kit (KK8504; Kapa Biosystems, Wilmington, MA, USA) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) to construct the NGS libraries of cfDNA and quantify the library concentrations, respectively. The libraries were pooled in equimolar amounts and sequenced using a NextSeq™ 550Dx sequencer (Illumina) with a 75-bp single-end loading method to obtain more than 20 million reads for each sample. Finally, the unqualified sequences were filtered to obtain target sequences using Trimmomatic v0.40, and Kraken2 v2.1.0 was used to automatically interpret and generate reports.

2.3. Serological test

To further verify the accuracy of the mNGS, we planned to send the patient's serum to the Center for Disease Control (CDC) for serological confirmation. The CDC replied that the test could not be performed because of nonendemic area of Q fever in China and lack of a valid certificate for serological testing reagents for *C. burnetii.* Therefore, the qPCR was adopted to validate our results.

2.4. Multiplex quantitative PCR(qPCR)

The mcfDNA used for qPCR was extracted from 500 μL supernatant from peripheral blood using the PathoXtract® WYXM03010S mcfDNA enrichment extraction kit (WillingMed, Beijing, China). Primer and probe sequences are listed in Table 1. All oligonucleotides

Table 1
Primers and hydrolysis probes for multiplex qPCR.

Target gene	Primer or prob	Primer and probe sequences (5' –3')	Purification	Product length (bp)
icd	*icd*primer-f	GACCGACCCATTATTCCCT	PAGE	133
	*icd*primer-r	CGGCGTAGATCTCCATCCA		
	probe-*icd*	CGCCCGTCATGAAAAACGTGGTC		
com1	*com1*primer-f	AAGCAATTAAAGAAAATGCAAAGAAATTAT		139
	*com1*primer-r	ACAGAATTCATGGCTTTGCAAT	PAGE	
	probe-*com1*	CACATTGATAATCGAAAAATTCAACCAATG		
IS1111	*IS1*primer-f	CGCAGCACGTCAAACCG	PAGE	146
	*IS1*primer-r	TATCTTTAACAGCGCTTGAACGTC		
	probe-*IS1*	ATGTCAAAAGTAACAAGAATGATCGTAAC		

Table 2
Report on *Coxsiella burnetii* Cases Detected by mNGS in the Last Five Years.

Year of publication	Age	Sex	exposured	clinical feature	mNGS	Specimen type of mNGS	Serological test	Diagnosis duration	treatment	follow-up
2022	43	M	N/A	fever, infective endocarditis (IE)	134(0.90 %)/214112 (98.96 %)	blood/tissue	Phase II IgG-pos	2days	DOX(100 mg, q12 h) HCQ (200 mg, tid)	more than one month without the occurrence of fever and without symptoms of chest tightness and breath holding
2022	77	M	5years ago, his neighbor tended cattle and sheep in the mountainous area 5 km (km) from where he lived.	only swollen and pain in his right knee; he had no fever, cough, or malaise. Prosthesis joint infection (PJI)	(6423 Copies/ml),3 d-mNGS(3669 reads),6 d-ptNGS (6423 Copies/ml),	joint fluid	IgM-Neg	2days	DOX(100 mg, bid) PROFLOX(400 mg,qd)/1 year.	6 month later, knee showed no signs of swelling,no relevant etiological tests were detected
2023	66	M	N/A	spinal infection:severe sharp low back pain, numb ness and lower limb weakness	Detected positive, reads was unknown	spinal lesions biopsy tissue	Neg	N/A	RFP (0.6 g qd), DOX (0.1 g bid) and LEV (0.4 g qd)/18 months	16month later MRI showed that vertebral edema signals disappeared and the graft of bone fused
2023	45	M	Y	fever, headache	38 reads	blood	N/A	2days	DOX (200 mg/day),(4days)	3 d, normothermia
	58	M	N	fever, skin eschar	32 reads	blood	N/A	3days	DOX(200 mg/day),(3days)	4 d, normothermia
	66	M	N	fever, muscle sore ness	336 reads	blood	N/A	4days	DOX(200 mg/day),(3days)	5 d, normothermia
	68	M	tick bite	fever	2 reads	blood	N/A	3days	DOX (200 mg/day),(9days)	6 d, normothermia
	70	M	Y	fever	16 reads	blood	N/A	8days	DOX (200 mg/day),(4days)	9 d, normothermia
2023	34	M	insect bites on the left lower two weeks prior	high fever, lethargy, pulmonary infection, and liver damage	79reads	blood	N/A	6 days	TC(250 mg, q6h)-7days	15days no recurrence of fever
2022	76	M	N/A	After operation2 years, the wound in the left iliac fossa was repeatedly ruptured and not healing	tissue-776 reads/plasma-negative	tissue and plasma	IgM-pos	N/A	DOX (0.1 g Q12h) and HCQ (0.2 g tid)- 1.5 years.	died two months after discharge due to massive hemorrhage
2022	48	M	buy fresh beef half a month ago	fever with chilly dizziness and headache	10reads, coverage 0.024 %	blood	antibody titers of IgG 1:256	4days	1.DOX (0.1 g, q12 h)-10 days. 5.DOX((0.1 g, bid)-6days.	1 month later recovered well
2022	51	M	NO	sneezing, a runny nose, fatigue and myalgia for 7 days, followed by high fever and severe headache for 4 days, aseptic meningitis	20 reads/Neg	Blood/cerebrospinal fluid	IgM-neg	3days	DOX(100 mg, bid)-5days	N/A
2022	8/99person		N/A	perioperative infective endocarditis (IE	(118–1,559,253)	tissue and boold	N/A	N/A	DOX, IMP cilastatin sodium, VAN	N/A

Note: hydroxychloroquine-HCQ, ceftriaxone-CRO , ceftazidime-CAZ, doxycycline-DOX, moxifloxacin hydrochloride-PROFLOX, vancomycin-VAN, levofloxacin-LEV, piperacillin-tazobactam-TZP, tetracycline-TC, rifampin-RFP, cephalosporin-CEPs, acyclovir- ACV , imipenem-IPM, N/A:no availabl

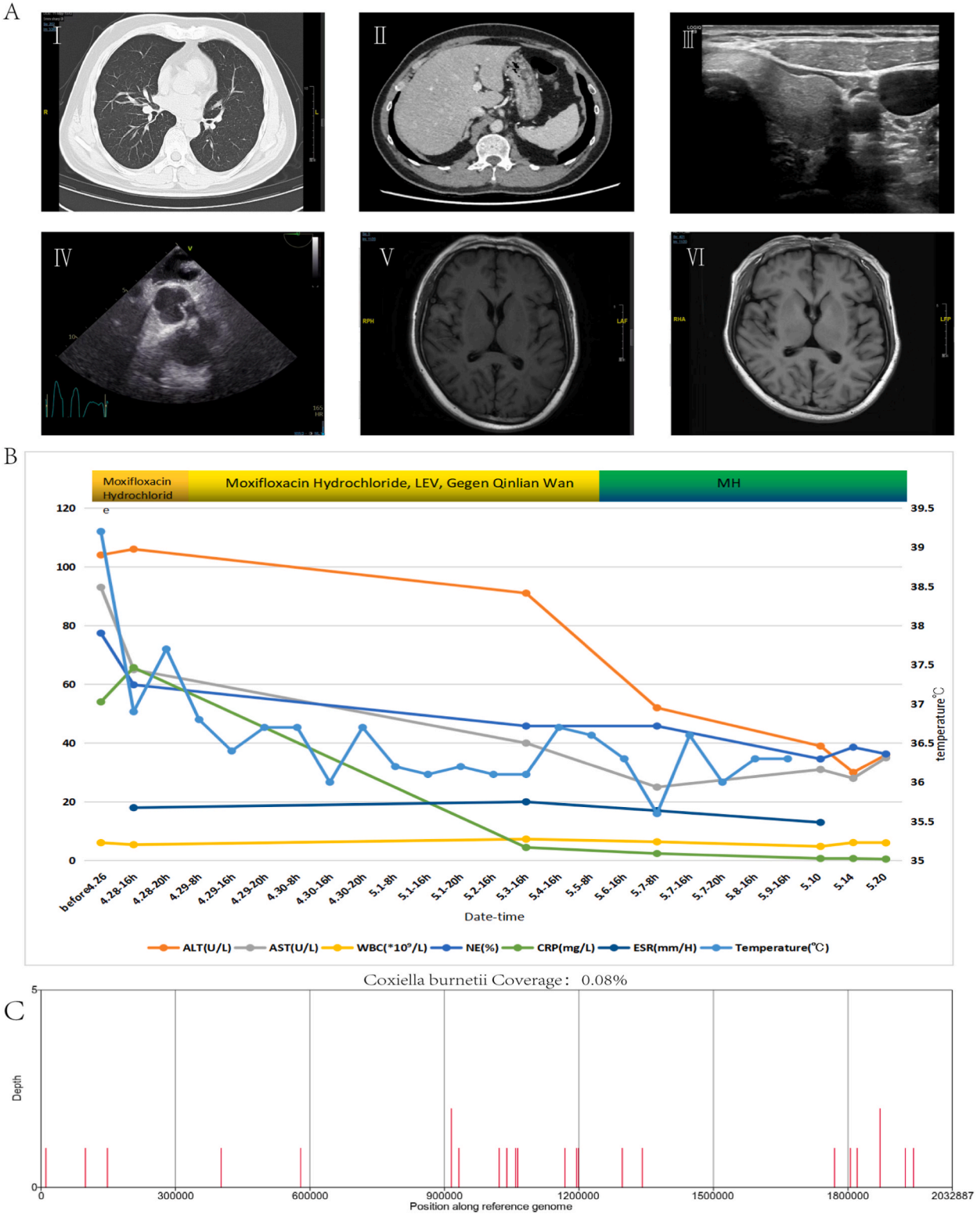

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Fig. 1. Laboratory, assistant examination, clinical test results after patient hospitalization.
A: Imaging examination. I: 26th April Chest CT. II: 2nd May Abdominal and pelvic CT. III and IV: 4th May Echocardiography and 7th May TEE before and after diagnosis. V and VI: 2nd and 10th May craniocerebral MRI before and after diagnosis. B: Dynamic monitoring of the patient's body temperature, and the level of CRP, ALT, AST et al. Besides, daily treatment schemes of the patient. C: The mNGS results show position along the reference genome of *C. burnetii* and a coverage of 0.08 %.
(Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell count; NE, neutrophils; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LEV, levoflfloxacin; MH, minocycline).

were synthesized by Tsingke (Beijing Tsingke Biotech Co., Ltd.). The final vloume for qPCR was 20 μL containing Luna Universal Probe qPCR Master Mix (BioLabs), 200 nM primers, and 100–300 nM hydrolysis probes. The amplifification conditions were as follows: 95 °C for 5 min, 50 thermocycles at 95 °C for 5 s and 60 °C for 35 s, followed by a fifinal incubation step at 50 °C for 30 s. We performed the qPCR on a ABI 7500 instrument and analysis was performed on the instrument's software systems v2.3.

2.5. Literature review

We searched PubMed using the keywords "mNGS" and "*C. burnetii*" for articles published in the prior five years, analyzing the infection characteristics of *C. burnetii* such as "age", "symptoms", and "treatment", excluding retrospective studies of mNGS, and identifying cases overlooked or misdiagnosed. The search results are presented in Table 2.

3. Case presentation

A 53-year-old man was admitted to our hospital on April 25, 2023, with unexplained uninterrupted fever for one week. After a business trip a week ago, he felt tired and developed uninterrupted fever to 39.2 °C, accompanied by chills and headaches. The temperature would decrease after self-administration of ibuprofen, but rising again a few minutes later. His medical history revealed that he had been cured of tuberculosis 40 years previously.

All laboratory tests failed to indicate the cause of fever, and no treatment was administered. Furthermore, the peripheral white blood cell (WBC) count was within the normal range. Due to persistent fever with chills and headaches, he visited the emergency department of Peking University People's Hospital on April 26, 2023. However, symptoms persisted after intravenous moxifloxacin (400 mg/250 ml) was administered once.

In addition, we also learned that the patient had been on a business trip to Sri Lanka, Saudi Arabia, and Iraq one week before the illness. Upon learning the special travel history, we further examined the patient. He did not visit areas where certain diseases were prevalent, was not bitten by mosquitoes or other insects, and had no symptoms similar to those of the surrounding population.

3 days post medical sought (April 28), the patient still had fever with headache and chills, and the laboratory examination revealed the following: WBC, neutrophil, and lymphocyte counts within normal limits; C-reactive protein (CRP), 54 mg/L (0–10 mg/L); erythrocyte sedimentation rate (ESR), 18 mm/h (0–15 mm/h); alanine aminotransferase (ALT), 106 U/L (9–50 U/L); aspartate aminotransferase (AST), 65 U/L (15–40 U/L); and lactate dehydrogenase (LDH), 311 U/L (109–245 U/L). Routine urinalysis revealed the presence of protein and occult blood. SARS-CoV-19 nucleic acid test results were negative. Chest CT revealed a few small benign or old nodules in both lungs.

A comprehensive analysis of the atypical fever symptoms, foreign travel history, and no relevant evidence of etiology of respiratory infection and no other special signs or toxic symptoms (suggesting dengue fever, hantavirus, or malaria). However, the laboratory indicators of infection were not significantly increased. After consultation, physicians and infectious disease specialists focused on some rare pathogens, recommending special investigative methods such as mNGS to identify rare pathogens, along with further testing to rule out rheumatological or neoplastic diseases. To clarify the cause, the patient was admitted to the hospital for further examination. 10 days post medical sought (May 5), mNGS reported a positive result for *C. burnetii*.

4. Results

4.1. Imaging

On April 26, Chest CT revealed a few small benign and old nodules in both lungs. On May 1, Brain MRI revealed multiple intracerebral cavity infarcts, cerebral white matter, and ethmoid sinusitis. Enhanced abdominal and pelvic CT revealed multiple enlarged hepatic hilar lymph nodes and hemangiomas. Thyroid ultrasonography revealed bilateral cystic and solid nodules. On May 7, the patient underwent transesophageal echocardiography; no clear thrombosis was identified in the left atrial appendage, and no cardiac neoplasms were observed. All above were shown as Fig. 1A I-VI.

4.2. Laboratory findings

Comprehensive laboratory findings were as follows: 1) All etiology-related tests for infectious diseases yielded negative results: influenza A/B nucleic acid test, SARS CoV-2 nucleic acid test, *M. pneumoniae* and *Chlamydia legionella* detection, and blood culture; 2) Related infection indicators: C-reactive protein (CRP), 54 mg/L (0–10 mg/L); erythrocyte sedimentation rate (ESR), 18 mm/h (0–15 mm/h); alanine aminotransferase (ALT), 106 U/L (9–50 U/L); aspartate aminotransferase (AST), 65 U/L (15–40 U/L); and lactate

dehydrogenase (LDH), 311 U/L (109–245 U/L). With the progress of treatment, relevant indicators gradually returned to normal, as shown in Fig. 1B.

4.3. mNGS and qPCR

After the above clinical laboratory and imaging results were found negative, the diagnosis was still in doubt. On May 5, mNGS identified DNA of *C. burnetii* with 20 reads, and the sequences measured is distributed in multiple locations in the genome of *C. burnetii* with the coverage of 0.08 % (Fig. 1C), so the results are credible. Furthermore, the qPCR showed a positive result to indicate that the nucleic acid of *C. burnetii* presented in the sample and comfirm the results of mNGS.

4.4. Diagnosis and treatment

The diagnosis of acute Q fever is based on the diagnostic criteria issued by the U. S. Centers for Disease Control and Prevention [5]. Symptomatic acute Q fever in adults, which occurs in approximately half of infected persons, is characterized by a wide variety of clinical signs and symptoms. After an incubation period of 2–3 weeks, the most common clinical manifestations are an isolated febrile syndrome or flu-like illness, including fever, fatigue, chills, and myalgia, which might occur in conjunction with pneumonia or hepatitis[3]. Most patients have normal WBC counts, increased ESR values, and hematuria, and increased CRP levels have been reported. The most common laboratory abnormality is increased liver enzyme levels. This nonspecific presentation is misleading, regardless of whether *C. burnetii* is the primary infection; therefore, determining epidemiological risk factors and conducting specific examinations are crucial in this context (see Fig. 2).

Considering the patient's atypical clinical symptoms (nothing special except fever and abnormal liver function), unclear records of outpatient treatment and special history of overseas travel, the clinicians focused on rare intracellular bacteria infections and have begun to experience medication intervention in patient's body temperature as well as attempting to suppress and kill pathogens internally. On admission, clinicians empirically ordered the traditional Chinese medicine Gegen Qinlian Wan (0.5 g orally, three times daily) combined with moxifloxacin (400 mg intravenously, once daily), levofloxacin (0.5 g orally, once daily) sequential therapy. After one week of treatment, the patient's temperature decreased and gradually returned to normal. At this time, the true cause of the patient's atypical fever was identified as *C. burnetii* (20 reads) using mNGS. In addition, we also conducted relevant imaging examinations on the patient, including chest CT, enhanced abdominal and pelvic CT, Brain MRI and transesophageal echocardiography. The results showed that no complications attributable to *C. burnetii* were observed, and regular re-examination was recommended. Thus, we diagnosed acute Q fever in this patient.

After the mNGS results were reported on May 5, the treatment was changed to oral minocycline 100 mg twice daily. Because the neutrophil count trended downward during treatment and the body temperature and other biochemical indicators normalized, discontinuation of medication was planned after two weeks. Regular follow-up was recommended, and the patient was discharged from the hospital. In a follow-up telephone conversation one month later, the patient and family members stated that they were in good condition and had no febrile symptoms. Later on, the patient was followed up regularly in the outpatient clinic of infection, but no relevant Q fever tests have been conducted and refused to undergo mNGS test again. In the telephone follow-up one year later, the patient reported that he had no physical discomfort in the past year.

5. Discussion

C. burnetii cannot be identified using Gram staining; however, it is stainable using the Gimenez method directly in clinical samples, and this is one reason the diagnosis is overlooked. *C. burnetii* can cause serious diseases and death and is transmitted in animals, including among humans. It can infect a large number of animals, such as mammals, birds, and arthropods, and can be excreted through milk, birth canal secretions, urine, feces, and semen [6]. Data show that it can replicate in high densities in placental trophoblastic cells, leading to abortion, stillbirth, and delivery of unhealthy offspring. Moreover, some animals are asymptomatic carriers [7]. Humans within 18 km can be infected via the wind by the inhalation of contaminated aerosols from affected animals

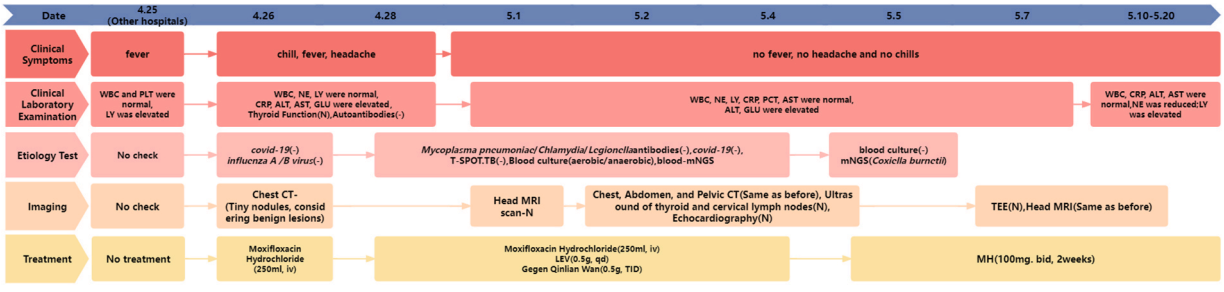

Fig. 2. Patient diagnosis and treatment timeline.
LY, lymphocyte; PLT, platelet; N: normal.

(mainly goats, sheep, and cattle) and their products (placenta, birth canal fluid, hides, wool, manure, and dairy products) [8]. For instance, from 2007 to 2010, a Q fever outbreak occurred in the Netherlands, resulting in over 4000 cases and 40,000 estimated exposed persons living around an infected livestock farm [9]. In another case, 138 patients with Q fever were diagnosed in Zhuhai, China, from December 2018 to March 2019, and most of the infected individuals lived or worked within 5 km of a slaughterhouse [10]. Moreover, some infections can be spread by foodborne and tickborne mechanisms [11]. Although anecdotal person-to-person transmissions have been reported less often, the main modes of transmission remain unclear [12].

In our case, the patient was healthy and had no history except for tuberculosis, which developed 40 years prior. At the initial onset of illness, he presented with flu-like manifestations (fever as high as 39.2 °C as the predominant sign, accompanied by chills and headache) and abnormal elevation of transaminases in peripheral blood. The first brain MRI (May 2, 2023) did not clearly indicate an infectious process, and the patient presented with bilateral ethmoid sinusitis associated with allergic rhinitis. On April 26, 2023, the first plain chest CT images showed a few tiny nodules in both lungs, which were consistent with those observed on the second scan (May 2, 2023) and were considered benign or old lesions. Enhanced CT of the chest, abdomen, and pelvis showed multiple enlarged lymph nodes at the hepatic hilum with significant enhancement, approximately 2.3 cm × 1.3 cm in size, which may be related to the abnormal aminotransferase levels. All etiology-related tests for infectious diseases yielded negative results, including influenza A/B nucleic acid test, SARS CoV-2 nucleic acid test, *M. pneumoniae* and *Chlamydia legionella* detection, and blood culture. At this moment, the clinicians had struggled to find direct evidence for the cause of the fever. They choose the latest detection technology mNGS to search for pathogens. The mNGS showed that 20 reads of *C. burnetii* have been detected in the patient's peripheral blood. Furthermore, the qPCR was adapted to verify the mNGS and showed a positive result consistent with the mNGS. As the aforementioned above, the patient's onset was insidious, and the laboratory tests after admission did not cover the relevant tests for *C. burnetii*. This is largely due to the fact that fewer cases of Q fever in China and the clinicians lack experience in diagnosing the disease. In addition, the current domestic clinical laboratories lack of routine detection reagents for *C. burnetii*, such as serological and PCR testing. Based on this case, we searched PubMed using the keywords "mNGS" and "*C. burnetii*" for articles published in the prior five years, analyzing the infection characteristics of *C. burnetii,* as noted in Table 2 [13–21]. It was found that all cases of Q fever were detected by mNGS, but most of them were clinically nonspecific except for varying degrees of fever. The disease was insidious and the source of infection was unclear, with only 4 cases were found to have a history of possible exposure, including 2 cases of possible contact with diseased animals [14,19] and 2 cases of insect bites [16,17]. Due to the small number of cases, gender and age distribution cannot provide effective clinical diagnosis and treatment assistance. Furthermore, the tissue or fluid from a patient's focal infection site is more efficient for mNGS than peripheral blood and is more likely to harbor suspected pathogens. Blood mNGS can only detect cell-free (cf) DNA of pathogens entering the bloodstream and cannot accurately determine the primary infection site. Moreover, the read counts are lower than those of the infected tissue or fluid. Therefore, we strongly recommend collecting tissues or body fluids from infected sites for mNGS to improve the credibility of the results.

In recent years, mNGS has been widely promoted in clinical practice, not only because of its broad coverage, unbiased nature, and non-assumption, but also because of its greatly shortened reporting time. The turnover time for traditional microbiological culture is 3–4 days, from sample collection, incubation, and development of visible colonies to bacterial identification and antibiotic sensitivity tests. mNGS, however, takes only hours and provides timely data on anti-infectives to the clinician, especially for slow-growing pathogens such as *Mycobacterium tuberculosis* [22]. A case series reported 29 patients with chronic sequelae of Q fever. After the acute phase, the detection of *C. burnetii* in peripheral blood decreased sharply using TaqMan-based PCR technology. However, the pathogens can persist in monocytes, placental cells, heart valves, liver, spleen, prostate, and bone marrow [23]. This is one reason why *C. burnetii* is difficult to detect using conventional methods. In addition, most infections related to Q fever were treated empirically before the pathogen was identified, which is a major drawback of microbial cultures, as observed in our case. Broadly speaking, prompt selection of mNGS is crucial for fever of unknown origin and difficult-to-culture pathogens in clinical microbiology laboratories. In our case, there were no actionable results from the clinical laboratory until the mNGS detected the DNA of *C. burnetii* in the patient's peripheral blood on May 5, 2023. However, the disadvantages of mNGS should not be ignored, it is too expensive to be routinely carried out in hospitals; the different type of specimens have a great impact on the results of mNGS. As listed in Table 2, the comparison of results between blood samples and tissue or body fluid samples shows that the number of reads does not reflect the severity of the disease; Overwhelming amount of host nucleic acid, reagent microbiome and contaminant nucleic acid can cause excessive interpretation of mNGS, leading to an increase in false positive rates.

C. burnetii is an obligate intracellular pathogen that is difficult to culture in conventional media. Accordingly, obtaining the results of the antibiotic susceptibility testing is an obstacle. This is another reason why Q fever is easily overlooked or misdiagnosed, leading to treatment failure and the emergence of drug-resistant strains. For decades, anti-infective treatments have remained in the exploratory stage. An increasing number of studies have focused on antibiotic sensitivity by using immunofluorescence assays, qPCR, and flow cytometry to detect intracellular *C. burnetii* [24]. The MIC values cannot be accurately measured *in vitro*, resulting in inefficient medication selection. Nevertheless, tetracyclines, fluoroquinolones, and macrolides have been selected by clinicians as the primary drugs against intracellular bacteria based on empirical approaches and literature reviews. Doxycycline is the most effective antibiotic against *C. burnetii*. However, the *in vitro* activity should not rely solely on susceptibility breakpoints, exposure of antimicrobial PK-PD targets to drugs at the site of infection, or varying tissue permeabilities. For example, doxycycline may be categorized as susceptible based on the current CLSI breakpoint (MIC $\leq$4 mg/L); however, the most effective value against *C. burnetii* was determined as $\leq$ 2 mg/L [25]. Chronic Q fever is a serious threat to humans, as drug-resistant (doxycycline MIC $\geq$8 mg/L) strains are emerging and causing death [26]. In addition, *C. burnetii* can reproduce in phagolysosome-like vacuoles because of its acidophilic properties. Alkalinizing agent (hydroxychloroquine) combined with quinolones and prolonged administration can restore the efficacy of doxycycline against *C. burnetii* and effectively reduce the recurrence rate of chronic Q fever. In our case, the antibiotic susceptibility tests are unable to

perform in our laboratory. The patient was treated empirically with intravenous moxifloxacin and oral levofloxacin for one week, then discharged with oral minocycline for two weeks. Afterwards, the patient was regularly followed up on the outpatient clinic of infection, and we also conducted telephone tracking. No adverse or unexpected events were found during telephone follow-up one year later.

A Q fever vaccine has been recommended for high-risk occupations related to livestock, particularly veterinarians, herders, and abattoir workers [27]. Studies have shown that the vaccine made from inactivated phase II *C. burnetii* generated high-avidity antibodies and robust T cell responses, without local allergic reactions, and contributed to a 50 % decline in Q fever incidence [28]. However, the vaccine has only been administered in countries with developed livestock industries such as Australia and Netherlands, and has not been rolled out worldwide [27,28].

6. Limitation

There are some limitations of the case: First, the patient has a history of traveling abroad, which makes a challenge to obtain the true source of infection and local epidemiology, and to screen for pathogens among his fellow travelers. Second, the domestic clinical laboratories lack valid certificates of serological reagents for *C. burnetii*, resulting in the inability to compare serological titers during the convalescent stage of patients; Third, mNGS is too expensive to be routinely carried out in hospitals, nor can it be used to monitor efficacy during Q fever treatment.

7. Conclusion

Q fever caused by *C. burnetii* is difficult to diagnose and has a poor outcome due to delayed treatment. *C. burnetii* belongs to intracellular bacterium that is challenging to cultivate routinely and conduct antibiotic sensitivity testing *in vitro*. In this context, mNGS is a significant tool for rapidly identifying pathogens and complementing conventional diagnostic methods when cultured-negative. In the future, mNGS will advance information on virulence and resistance of pathogens, providing faster and more effective antibiotic options for patients.

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Ethics approval and consent to participate

This study was approved by the research ethics board at Peking University People's Hospital (ID: 2023PHB149-001). We followed up the case after the patient was discharged from the hospital, and we have obtained verbal consent from the patient by telephone, and promised that only the patient's laborator-related examination, including the publication of any potentially identifiable images or datas included in this article, without any privacy concerns of the patient.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Qiaoli Xu: Writing – review & editing, Writing – original draft, Resources, Formal analysis, Conceptualization. **Wenyan Han:** Writing – review & editing, Formal analysis, Data curation. **Yihua Cai:** Writing – review & editing, Formal analysis, Data curation. **Yuyao Yin:** Software, Resources, Data curation. **Yifan Guo:** Software, Resources, Data curation. **Hongbin Chen:** Writing – review & editing, Validation, Funding acquisition, Formal analysis, Conceptualization. **Hui Wang:** Writing – review & editing, Validation, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with people or organizations that can inappropriately influence our work, there is no professional or other personal intersest of any nature or kind in any product, service and/or company that could be construed as influencing the position persented in, or the review of, the manuscript entitled.

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