CASE REPORT

Diagnosis of *Coxiella burnetii* Prosthetic Joint Infection Using mNGS and ptNGS: A Case Report and Literature Review

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Background: *Coxiella burnetii* (*C. burnetii*) is the causative agent of Q fever and is found worldwide; however, prosthetic joint infections caused by *C. burnetii* are rarely seen. Because of advances in molecular diagnostic techniques, prosthetic joint infection (PJI) caused by *C. burnetii* can now be diagnosed.

Case Presentation: A 77-year-old male who had undergone total knee arthroplasty had a displaced prosthesis and periprosthetic osteolysis; he had no obvious signs of infection, and microbiological culture was negative. However, *C. burnetii* was detected by metagenomic next-generation sequencing (mNGS) and pathogen-targeted next-generation sequencing (ptNGS). Finally, polymerase chain reaction (PCR) confirmed the diagnosis of *C. burnetii* prosthetic joint infection (PJI). After revision surgery (one-stage revision) and oral antibiotics (doxycycline and moxifloxacin hydrochloride), the patient's symptoms disappeared, and he regained the ability to walk. During the 6-month follow-up, the patient's knee showed no signs of swelling, pain or the recurrence of infection, and he experienced no significant complications. We also present a review of the literature for other cases of *C. burnetii* PJI.

Conclusions: The symptoms of *C. burnetii* PJI may be different from those of Q fever, which may lead to misdiagnosis. mNGS and ptNGS may be helpful for the identification of *C. burnetii*. Once the diagnosis of *C. burnetii* PJI is confirmed, doxycycline in combination with a fluoroquinolone can be effectively administered after revision surgery.

Key words: *Coxiella burnetii*; Metagenomic next-generation sequencing; PCR-based targeted next-generation sequencing; Prosthetic joint infection

Introduction

Coxiella burnetii is a strictly intracellular, gram-negative bacterium[1] that can be transmitted to humans from arthropods, mainly through the air or insects from sheep, cattle and goats. Q fever, which is one of the causes of endocarditis, is caused by C. burnetii.[2] Patients with Q fever present with flu-like symptoms, including fever, headache, and myalgia, but some patients can have no obvious symptoms.[1,3] C. burnetii osteomyelitis is rare, and C. burnetii has been reported to account for only 2% of the cases of Q fever found in France.[1] Prosthetic joint infection (PJI) is a catastrophic complication of arthroplasty. The identification of the causative pathogen is the gold standard for the diagnosis of PJI and can inform the administration of antibiotics.[4] There are few reports related to PJI caused by C. burnetii, which may be due to the difficulty of detecting C. burnetii by conventional culture techniques. Here, we report a case of C. burnetii PJI that was confirmed by molecular diagnostic techniques.

Case Report

A 77-year-old man presented to our department with persistent pain in and swelling of his right knee for

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2 years. Bilateral total knee arthroplasty was performed 17 years prior to presentation due to severe degeneration. Two years prior to presentation, he began experiencing right knee pain and swelling, and 3 months prior to presentation, he lost the ability to walk. Over the 2 years prior to presentation, he did not suffer from fever, chills, cough or general discomfort, and he took oral painkillers intermittently without antibiotics. It is worth noting that 5 years prior to presentation, his neighbor tended cattle and sheep in the mountainous area 5 kilometers (km) from where he lived. He had no history of traveling abroad.

His BMI was 23.18 kg/m^2. He had a history of hypertension, but his blood pressure was well controlled. He had no other underlying medical conditions. His skin was intact, and his skin temperature was normal, but his right knee was swollen. An analysis of inflammatory marker levels found that his C-reactive protein (CRP) level was 15.20 mg/L (0–8), and his erythrocyte sedimentation rate (ESR) was 19 mm/h (0–38). Single-photon emission CT revealed an abnormal radiolucent shadow around the right knee joint (Fig. 1A). X-ray revealed a displacement of the right knee prosthesis with severe osteolysis around the prosthesis (Fig. 2B–D). Echocardiography findings did not suggest endocarditis. Knee joint puncture was performed before surgery, and 10 ml of yellow, slightly cloudy fluid was aspirated. The synovial fluid WBC count was 1816/μL, and the synovial fluid PMN% was 33.8%. The synovial fluid was subjected to routine microbiological tests, including acid-fast staining, Gram staining, bacterial culture (aerobic and anaerobic) and fungal culture, but the results of all of these tests were negative (Fig. 2A). Some specimens underwent mNGS testing (the details of which can be found in the supplementary material), and the results after 48 h revealed *C. burnetii* infection (Fig. 2B, Table 1). He was considered preoperatively for possible infection according to the 2018 International Consensus Meeting (ICM) criteria.[5]

Although we recommended that the patient undergo a two-stage revision surgery, we ultimately performed a

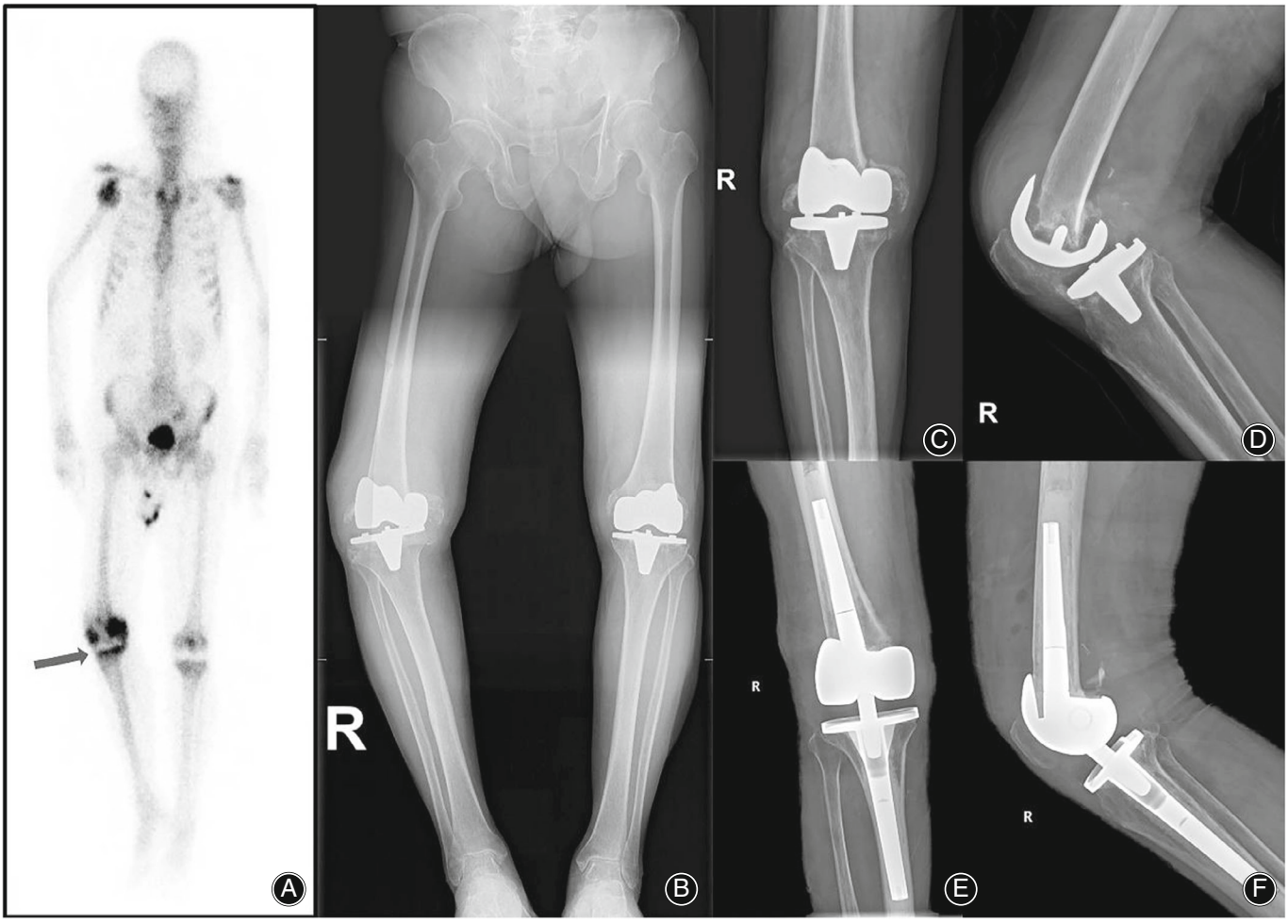
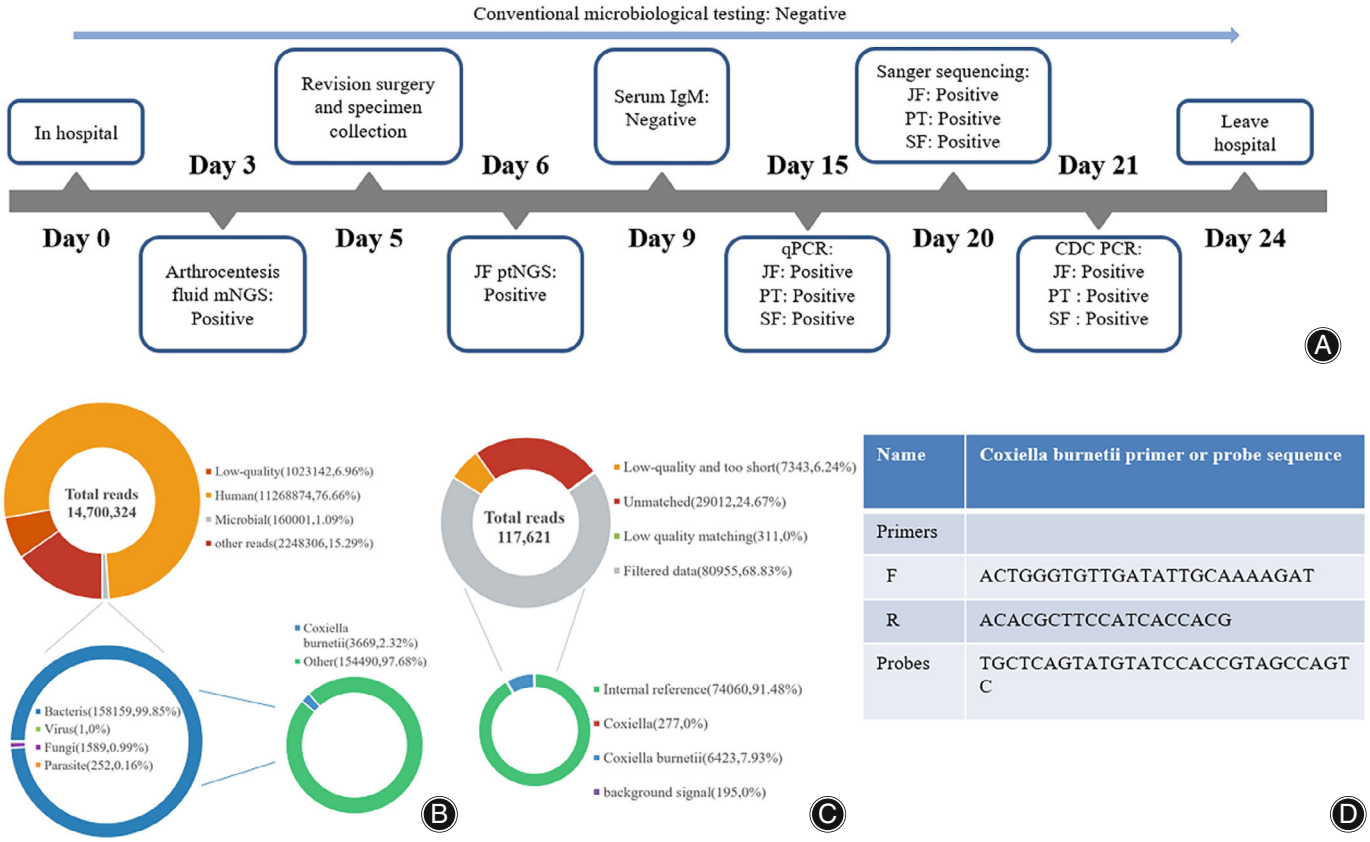


Fig. 1 (A) SPECT showed an abnormal radiolucent shadow around the right knee joint (at the arrow), (B–D) X-ray before operation revealed prosthesis displacement and peripheral osteolysis, (E, F) X-ray after operation



Name	Coxiella burnetii primer or probe sequence
Primers	
F	ACTGGGTGTTGATATTGCAAAAGAT
R	ACACGCTTCCATCACCACG
Probes	TGCTCAGTATGTATCCACCGTAGCCAGTC

Fig. 2 (A) The timeline of pathogen investigations, (B) *Coxiella burnetii* was detected by mNGS of joint fluid before operation (3669 reads), (C) *Coxiella burnetii* was detected by ptNGS of joint fluid during operation (6423 Copies/ml), (D) *Coxiella burnetii* primer or probe sequences.

one-stage revision at his request. For the one-stage revision, in brief, the original prosthesis was removed, the knee joint was completely debrided and finally a new prosthesis was directly implanted into the joint. During the revision, inflammatory granulation tissue was seen in the capsule, and the joint fluid was yellowish-brown. Intraoperative histopathology revealed seven neutrophils at high magnification (×400) in one specimen and fewer than five neutrophils in the other four specimens. Intraoperatively, joint fluid (JF) and prosthetic tissue (PT, five different sites) were collected, and the removed prosthesis was sonicated to obtain sonication fluid (SF). Conventional microbiological tests were performed on all types of specimens, and the results were all negative. We performed pathogen-targeted next-generation sequencing (ptNGS, a multiple PCR-based targeted NGS technique;[6] the details of which can be found in the supplementary material)

TABLE 1 Microbes detected by mNGS and ptNGS

	mNGS		ptNGS	
Parameters	Microbe	Reads	Microbe	Copies/ml
Pathogenic microorganisms	*Coxiella burnetii*	3669	*Coxiella burnetii*	6423
Background microorganisms	*Staphylococcus arlettae*	48	*BK virus*	8
	Clostridium beijerinckii	121	*human polyomavirus V*	2
	Morganella morganii	67	*Parvovirus type 18*	7
	Klebsiella aerogenes	5	*Necator americanus*	4
	Achromobacter xylosoxidans	23		
	Candida parapsilosis	4		

Abbreviations: JF: joint fluid, PT: prosthetic tissue, SF: sonication fluid, mNGS: metagenomic next-generation sequencing, ptNGS: pathogen-targeted next-generation sequencing.

TABLE 2 Summary of patients with prosthetic joint infections associated with *Coxiella burnetii*

Year of publication	Age, sex	Exposurerisk factors	Involved joint	Clinical symptoms	Diagnosis	Antibiotic regimen/Duration	Outcome
2021[14]	65, F	Contact with cattle	Hip (THA)	Pain Fever	PCR Serology	Ofloxacin and Rifampin/18 months	No evidence of relapsed infection
2019[15]	62, M	No	Hip (Revised THA)	Pain Swelling	PCR 16 S rDNA Serology	Doxycycline and hydroxychloroquine/18 months	Prosthesis loosening
2018[16]	72, F	No	Knee (TKA)	Pain Swelling Loosening	PCR Serology	Hydroxychloroquine and doxycycline/18 months	Not described
2017[17]	64, F	Visited agricultural areas	Knee (TKA)	Pain Swelling Night sweats Headaches Cough	PCR Serology	Doxycycline and hydroxychloroquine/24 months	No evidence of relapsed infection
2013[18]	56, M	No	Knee (Revised TKA)	Pain Swelling	PCR Serology Culture	Ciprofloxacin and doxycycline, then trimethoprim-sulfamethoxazole and doxycycline, and then hydroxychloroquine and doxycycline/Not described	No evidence of relapsed infection
2013[19]	63, F	Contact with sheep	Hip (Revised THA)	Pain	PCR 16 S rDNA Serology	Doxycycline and hydroxychloroquine/Not described	Spacer explantation
2012[19]	84, M	Not described	Hip (THA)	Pain Fever	Serology	Doxycycline and hydroxychloroquine/Not described	No evidence of relapsed infection
2012[19]	82, M	Not described	Hip (Revised THA)	Asymptomatic	PCR Serology	Doxycycline and hydroxychloroquine/Not described	No evidence of relapsed infection
2012[19]	60, F	Not described	Hip (Revised THA)	Pain Fever	PCR 16 S rDNA Serology	Doxycycline and hydroxychloroquine/Not described	No evidence of relapsed infection

Abbreviations: TKA, Total knee arthroplasty; THA, Total hip arthroplasty.

on the intraoperative samples, and *C. burnetii* was detected after 14 h (Fig. 2C, Table 1). Finally, we performed qPCR and PCR combined with Sanger sequencing, and both detected *C. burnetii* (primer sequences in Fig. 2D). At this point, the patient was diagnosed with a PJI. We also performed a serological test for *C. burnetii* IgM antibodies, but the result was negative.

The patient was not started on antibiotics until the day of surgery. He was started on intravenous vancomycin (1 g/12 h) combined with ceftazidime (1 g/12 h) half an hour before surgery. Two days later, after obtaining the ptNGS results, we conducted an multiple disciplinary team (MDT) meeting (including the orthopedic, infectious disease, microbiology and pharmacy departments), and the antibiotic regimen was changed to oral doxycycline (100 mg twice a day) and moxifloxacin hydrochloride (400 mg once a day) for a duration of 1 year.

The patient began to walk with assistance on the second day after the operation. The postoperative X-ray is shown in Fig. 1E,F. The levels of inflammatory markers returned to normal before discharge. During the 6-month follow-up, the patient's knee showed no signs of swelling, pain or the recurrence of infection, and he experienced no significant complications.

Discussion

Clinical Manifestations and Epidemiological Investigations

PJI caused by *C. burnetii* is rare, but its incidence may be underestimated. With the progress in molecular diagnostic techniques, difficult-to-culture bacteria are attracting increasing attention. Here, we report the case of a patient with *C. burnetii* PJI detected by mNGS and ptNGS. Our PCR tests on several samples finally confirmed that *C. burnetii* was the causative pathogen. In this case, the patient had only swollen and pain in his right knee; he had no fever, cough, or malaise. According to Table 2, a majority of patients with *C. burnetii* PJI also showed localized swelling and pain, suggesting a nonspecific clinical presentation. In this case, patient exposure to *C. burnetii* was a possibility since there was a cattle and sheep breeding site 5 km away from his

home 5 years prior to presentation. Tissot-DuPont *et al.* suggested that aerosols containing *C. burnetii* could spread over 30 km;[7] however, we were unable to pinpoint that this was the source of his infection.

Diagnosis of *C. burnetii* PJI

According to the 2018 ICM,[5] only CRP levels and intraoperative histopathology findings support the diagnosis of suspected PJI. Before revision surgery, investigations on the causative pathogen should be performed in patients with suspected PJI; however, in this case, the microbiological results of the patient's preoperative and intraoperative specimens were negative. In some cases, the use of antibiotics can lead to negative cultures,[8] but in this case, the patient did not take antibiotics before surgery, which shows the limitations of traditional methods for detecting *C. burnetii* (or atypical pathogens). This also illustrates that the diagnosis of *C. burnetii* PJI remains a challenging task for physicians. This type of infection requires careful patient evaluation and extensive workup, including unbiased sampling for mNGS, to detect the causative microorganism.

Serological testing is the predominant method for the detection of *C. burnetii*.[1] Similar to previous reports (Table 2), the results of tests for the detection of IgM in this case were negative, indicating that serological testing for *C. burnetii* PJI may not be sensitive (Certified IgG detection was not available in China). Realtime quantitative polymerase chain reaction (qPCR) is also an effective tool for the diagnosis of *C. burnetiid*;[1] however, it is also difficult for clinicians to include *C. burnetii* in routine testing in non-Q fever endemic regions. In this case, mNGS detected *C. burnetii* preoperatively, and unbiased sampling for mNGS avoided a missed diagnosis. Moreover, intraoperative samples underwent rapid detection using the ptNGS method. The specific sequences of *C. burnetii* were covered in the osteoarticular infection panel we applied, which covered sufficient pathogenic species. The detection time and cost of ptNGS were lower than those of mNGS, and ptNGS addressed the inherent limitations of mNGS,[9] as it is known that excessive consumption of sequencing resources by human-derived nucleic acids reduces pathogen detection sensitivity and improves theoretical detection sensitivity.

However, neither ptNGS nor mNGS currently have standards for the interpretation of results, and they cannot be used to confirm the diagnosis of PJI but only as adjunctive methods for the screening for causative organisms.[9] For atypical pathogenic bacteria, when screening with mNGS or ptNGS, it may be an effective strategy to confirm the diagnosis of PJI by selecting specific culture conditions based on the characteristics of the pathogenic bacteria[10] or by PCR validation. In this case, our final diagnosis of PJI was based on the PCR results of several different specimens. The diagnosis of PJI still requires multi-disciplinary cooperation, and each microbiological result should be analyzed on a case-by-case basis.

Antibiotic Regimen of *C. burnetii* PJI

Despite the risk of drug resistance, doxycycline and fluoroquinolones[11–13] remain the most effective drugs for *C. burnetiid* infection, and we developed an antibiotic regimen of oral doxycycline + moxifloxacin hydrochloride after an MDT meeting and recommended its use for more than 1 year. At the most recent 6-month follow-up, the patient still had no symptoms of PJI and was able to walk normally. However, our follow-up was still not long enough, which is one of the limitations of this study. Studies with longer follow-ups and multicentre case–control studies are needed in the future.

Conclusion

Unlike Q fever, the clinical presentation of *C. burnetii* PJI may be atypical. *C. burnetii* should be considered when culture-negative PJIs are encountered. Molecular diagnostic methods are important tools for the diagnosis of *C. burnetii* PJI, and among these methods, mNGS and ptNGS may be effective in screening for pathogens. Once *C. burnetii* PJI is diagnosed, doxycycline combined with fluoroquinolones may be effective on a background of revision surgery.

Ethical Approval and Consent to Participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the First Affiliated Hospital of Fujian Medical University Ethics Committee. (Approval No: MTCA, ECFAH of FMU [2015] 084-2). Participant was informed about the study and consent to participate. The written consent to publish this information was obtained from study participants.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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Author Contributions

Changyu Huang and Haiqi Ding led the writing of the manuscript. Zida Huang and Wenming Zhang developed the initial concept and framework for the manuscript and oversaw the drafting of the manuscript. All authors contributed to the content, drafting, and critical review of the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article on the publisher's web-site:

Appendix S1. Supporting Information.

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