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Case report

The first human case report of molecularly confirmed co-infection of *Brucella melitensis* and *Coxiella burnetii:* A case report

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ABSTRACT

Co-infection with *Brucella melitensis* and *Coxiella burnetii* has been rarely reported. To date, there are only two co-infection case reports from Croatia and China which diagnosed the infections mainly through the use of serological tests. In this report, we present the first case of molecularly confirmed *B. melitensis* bacteremia and *C. burnetii* spondylodiscitis co-infection in a goat dairy farmer who presented with lumbosacral spondylodiscitis and bilateral psoas abscesses. From the blood culture, *B. melitensis* was identified by using 16S rRNA gene sequencing and specific PCR. Lumbar bone tissue was found to be positive for *C. burnetii* using multiplex real-time PCR and was confirmed with a positive result from conventional PCR which detected the infection through the identification of the *IS1111* gene. The patient's condition improved after decompressive laminectomy was performed and administration of antibiotics regimen: intravenous gentamicin, oral rifampicin, and oral doxycycline. From our case, it is important to raise awareness of this underreported co-infection with multiple zoonotic diseases, especially Q fever and brucellosis, which share the same exposure risk. Moreover, we also emphasize the use of advanced molecular techniques to improve the diagnostic efficiency and reduce the use of time-consuming procedures among patients who are continuously exposed to such risk factors in areas with high seroprevalence of these zoonotic diseases.

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1. Introduction

Brucellosis and Q fever are recognized as occupational zoonoses caused by intracellular gram-negative bacteria, *Brucella* spp. and *Coxiella burnetii*, respectively [1]. These diseases share similar source of infection, route of transmission, clinical manifestations, and hosts, particularly among people involved in handling infected livestock such as veterinarians, abattoir workers, and dairy farmers. Transmission can occur through the inhalation of infectious aerosol particles, consumption of unpasteurized dairy products, or contact with milk, urine, feces, or vaginal secretions from infected animals. Some people who are infected with these zoonotic infections are asymptomatic. However, others may have undifferentiated febrile illness during the acute phase of the infection. Chronic infection can present as endocarditis, hepatitis, osteomyelitis, spondylitis, or central nervous system (CNS) infection [2]. Nevertheless, co-infection with brucellosis and Q fever is rarely reported. When we did a literature search, we found only two cases of this co-infection reported from Croatia and China [1,3]. Both of them detected brucellosis using microbiological culture, but the diagnosis of Q fever was diagnosed based only on serology methods. In this report, we utilized microbiological culture and molecular methods to confirm co-infection of brucellosis and Q fever, distinguishing it from the previous cases.

2. Case presentation

A 64-year-old Thai male patient without known pre-existing comorbidities, was hospitalized at King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand, during September 2022. He was a goat dairy farmer from Chainart province, Northern Thailand. He had suffered from progressive chronic lower back pain without fever for a year. No previous trauma history was recorded. Pain was aggravated by motion and partially relieved with nonsteroidal anti-inflammatory drugs (NSAIDs). However, his symptoms gradually worsened over six months. The pain began immediately upon awakening and disturbed his work and daily life. He received a primary diagnosis of spinal stenosis based on the MRI of the lumbosacral spine and was treated with gabapentin and NSAIDs by an orthopedist. Despite the use of multiple analgesics, his lower back pain persisted; then, he developed tightness in his right hip and inner thigh just a few weeks prior to admission. He did not experience headaches, fatigue, loss of appetite, or weight loss. According to the epidemiological investigation, the patient had been a goat dairy farmer for more than two years. He had assisted in the birthing of the goats many times, without proper protection. However, he neither consumed goat milk nor ate raw goat meat. Some of his goats experienced abortions of unknown cause in the past year, but he did not report these incidents to the Department of Livestock Development for an investigation. During admission, he was afebrile with normal vital functions. He had tenderness at the spinal and paraspinal areas of L4–L5 levels. Neurological examination was unremarkable. Lymphadenopathy, testicular mass, and abnormal skin lesions were absent. Furthermore, abdominal examination revealed no hepatosplenomegaly. The examination was otherwise apparently normal.

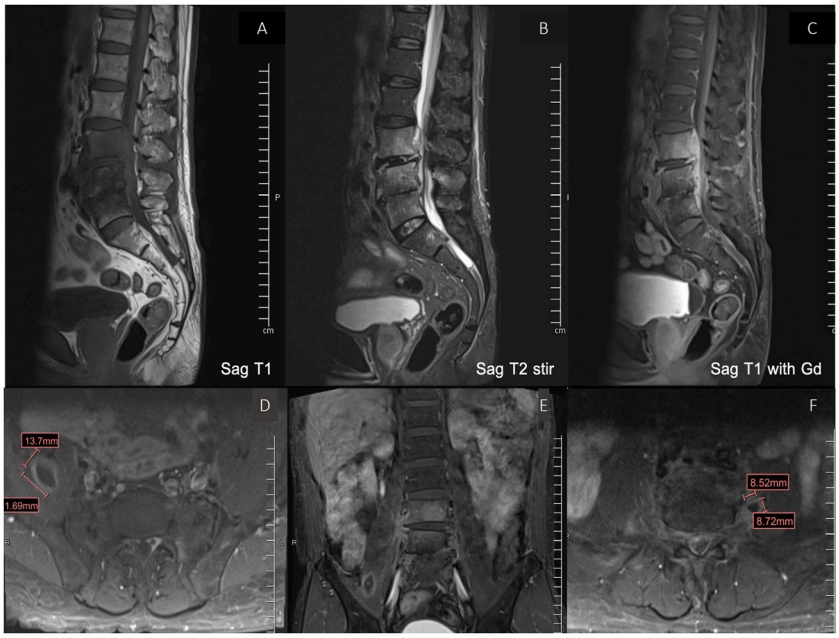

Fig. 1. Lumbosacral spine MRI A-C) Spondylodiscitis involvement of L3-S1 vertebra and L3/4 disc with tiny abscess at the L3–L5 ventral epidural space D-F) Rim enhancing abscesses involving the right psoas muscle at the L5/S1 level and left psoas muscle at L4/L5 level measuring 1.4. x1.7 × 2.5-cm and 0.9 × 0.9 × 1.0-cm., respectively.

2.1. Laboratory and radiological investigations

White blood cell (WBC) count, liver function test and serum creatinine showed no obvious abnormalities. In addition, the anti-HIV antibody test was negative. A chest X-ray did not reveal any abnormalities. A repeated MRI of the lumbosacral spine showed new spondylodiscitis involving L3-L4, L4-L5, and L5-S1 levels, together with phlegmon and small abscesses in the ventral epidural space at L3-L5 (Fig. 1(A–C)). Additionally, the MRI showed the right and left psoas abscesses measuring 1.4 × 1.7 × 2.5 cm and 0.9 × 0.9 × 1 cm, respectively (Fig. 1(D–F)).

2.2. Clinical course and definite diagnosis

He underwent a lumbar decompressive laminectomy with pedicle fusion due to uncontrollable pain. Intraoperative findings showed no obvious discharge. Histopathology of the bone tissue showed no granuloma or tumor cells in the bone and cartilaginous tissue. For the bone tissue microbiological test, no organisms were detected by the conventional culture but through multiplex real-time PCR (Fast Track Diagnostics (FTD)™ tropical fever Africa kit, Fast Track Diagnostics Ltd., Siemens Healthcare Diagnostics Inc.), *C. burnetii* was detected which was then confirmed by conventional PCR targeting the *IS1111* gene of *C. burnetii* (Fig. 2). For conventional PCR method, an *IS1111* plasmid was used as a positive control. The primers for the *IS1111* gene [4] are presented in Table 1. The thermocycling conditions were as follows: 98 °C for 30 seconds, followed by 35 cycles of 98 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 15 seconds, with a final step at 72 °C for 2 minutes. The patient's PCR product for *IS1111* was entered into the GenBank (GenBank accession number PP336779) which was 99.32 % identical to a sequence of *C. burnetii* when compared against the sequence from the National Center for Biotechnology Information (NCBI) database (Supplementary material 1.).

Furthermore, two blood cultures taken from two different peripheral veins were cultivated using an automated blood culture system (BACTEC™ FX). One of the blood cultures flagged positive at 102.25 hours for small gram-negative coccobacilli (Fig. 3). The bacteria had tiny smooth whitish colonies which grew on blood agar and chocolate agar plates. Although the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) (VITEK® MS) identified the organism as *Brucella* spp., it failed to specify the species. Therefore, the isolate was subjected to 16S rRNA gene sequencing, which was blasted against the National Center for Biotechnology Information (NCBI) database, resulting in a 100 % identical match with *Brucella* spp. (Supplementary material 1.). Furthermore, multiplex PCR targeting loci BMEI and BMEII (Table 2). Reference [5] confirmed that the bacterium was *B. melitensis*. Unfortunately, histopathology of the bone tissue could not confirm *B. melitensis* by direct immunofluorescent antibody (DIFA) or immunohistochemistry tests due to their unavailability in both our center and Thai Institute of Pathology. All of the mentioned results strongly supported the diagnosis of *B. melitensis* bacteremia and *C. burnetii* spondylodiscitis co-infection in this patient.

Serological tests were done after treatment because the lab test was not available at the time of diagnosis. When it was convenient for the patient to come to the hospital, paired sera were taken on day 30 and day 60 after treatment. Enzyme-linked immunosorbent assay (ELISA) for *Brucella* IgM and IgG were both positive at day 30. On the other hand, indirect immunofluorescent assay (IFA) for *C. burnetii* IgM and IgG were all negative at day 30 and day 60.

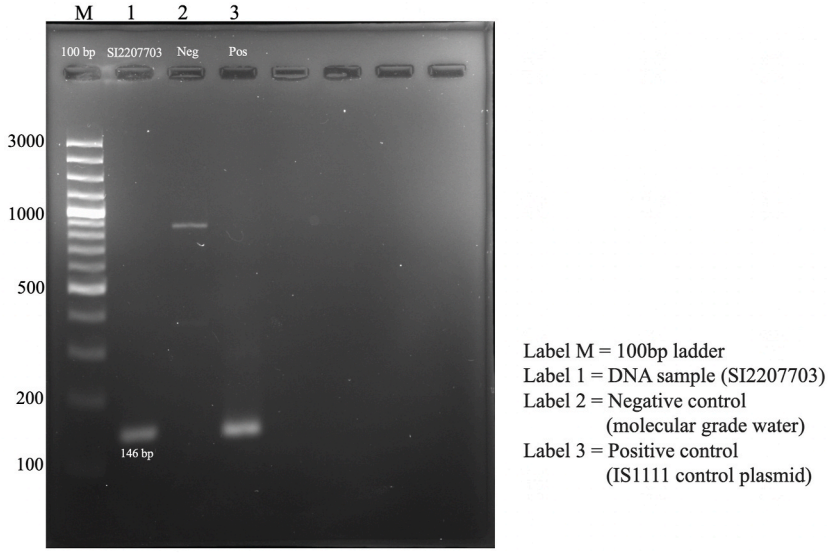

Fig. 2. Agarose gel electrophoresis of amplified fragment of *C. burnetii IS1111* gene (The non-adjusted gel image was provided as Supplementary material 2.).

Table 1
Primers for conventional PCR for *C. burnetii*.

Primer	Designation	Primer sequence (5′–3′)
Target *IS1111*		
Forward primer	IS1pri_f	CGCAGCACGTCAAACCG
Reverse primer	IS1pri_r	TATCTTTAACAGCGCTTGAACGTC

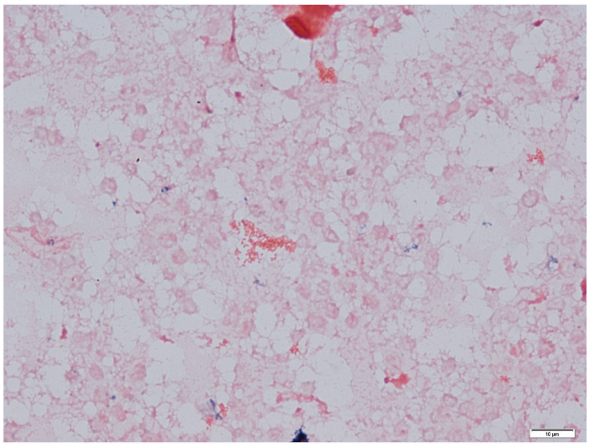

Fig. 3. Gram stain of *B. melitensis* from blood culture shows that there are small gram-negative coccobacilli (1000×).

Table 2
Coding sequence (CDS) for multiplex PCR to identify species of *Brucella* spp.

CDS names	
BR0	BR0953
BMEI	BMEI10752, BMEI1435, BMEI1436, BMEI0535, BMEI0536, BMEI0997 BMEI0998
BMEII	BMEII0987, BMEII0428, BMEII0843, BMEII0844

2.3. Treatment

The induction treatment regimen consisted of intravenous gentamicin 160 mg/day, oral rifampicin 900 mg/day, and oral doxycycline 200 mg/day for seven days. Upon discharge, oral rifampicin 900 mg/day, doxycycline 200 mg/day, and hydroxychloroquine 600 mg/day were prescribed as a continuous regimen. After a two-month period, the patient showed clinical improvement, and a lumbosacral spine MRI confirmed regression of both bilateral psoas and epidural abscesses, along with improved spondylodiscitis. Maintenance medications were continued with MRI monitoring.

3. Discussion

Brucellosis, a zoonotic infection caused by *Brucella* spp. [6], has been increasingly reported in humans in Thailand, with 238 cases documented between 2003 and 2019 by the Bureau of Disease Control and Veterinary Services. In contrast, Q fever, caused by *C. burnetii,* was seldom confirmed, despite having similar animal reservoir and transmission route. The true prevalence of Q fever remains unknown in Thailand. First clinical case series of acute Q-fever conducted in 2003, serological tests were done among 678 acute febrile patients, which were revealed nine cases fulfilled the diagnostic criteria [7]. National experts estimated it to be around 0.5 % among cases with acute undifferentiated fever and believed that it was underreported because the physicians did not suspect the patients to have this infection so it was not investigated [8].

Moreover, co-infection with *B. melitensis* and *C. burnetii* had never been documented in Thailand. Nevertheless, we found only two case reports of this co-infection from Croatia and China. In 2017, a 30-year-old male agriculturist in Croatia had a history of direct contact with sheep and consumed unpasteurized dairy products. He presented with significant weight loss and subacute fever with arthralgia. Brucellosis and acute Q fever were diagnosed by positive blood cultures of *B. melitensis* and *C. burnetii* serological tests, respectively. He was successfully treated with 6 weeks of doxycycline and rifampicin [3]. The second case was a 49-year-old shepherd from China. He had a high-grade fever after an open fracture at the left ulnar. After he had the symptoms, he went to the hospital for treatment. Blood was collected from the patient and subjected to blood culture. After 72 hours of incubation, there were growths in the blood culture. These colonies were collected and later were identified as *B. melitensis* by mass spectrometry. As for Q fever, it was

diagnosed by serological IFA. From the IFA, a positive IgM was detected. The patient was treated with 4 weeks of doxycycline and rifampicin [1].

Our patient had similar symptoms as the two previously reported cases. All cases were occupationally exposed individuals. However, the clinical presentation of Q fever were different. In the two prior cases, they had acute Q fever whereas our case had chronic Q fever. Notably, the diagnoses in both previous case reports relied on positive cultures of *B. melitensis* and positive serology tests for *C. burnetii* infection. In contrast, our case was diagnosed with brucellosis based on positive culture colonies with molecular identification to species level, whereas Q fever was diagnosed via molecular methods from a direct specimen. Unfortunately, we could not identify both organisms using the same clinical specimen. This may be because the bone tissue (from the posterior site via laminectomy) is not typically the site for brucellosis infection which usually involves the discs. Using laminar bone tissue for the diagnosis of brucellosis infection can lead to false negative result.

Moreover, for our patient, the serology test for *C. burnetii* was not indicative of chronic Q fever. The explanation for this is that the serology test was not performed immediately but after 30 and 60 days post treatment. By that time, the *C. burnetii* had already seroreverted. However, the CDC's surveillance case definition for chronic Q fever includes laboratory criteria such as the use of PCR to detect *C. burnetii* DNA in the clinical specimens [9]. For our patient, we used the PCR method to detect *C. burnetii*. Although we found a thin band sized nearly 10,000 bp on negative control during PCR test that might presumably result from subtle genomic DNA contamination from other source. It would not relevant to targeting band sized 146 bp of *C. burnetii* amplicon (Fig. 2.)

Since the PCR test has a high sensitivity, we were concerned whether the test was false positive. Hence, we reviewed the patient's history and contact with potentially infected goats to ascertain whether the detection of *C. burnetii* in the bone tissue was indeed positive or not. However, we reported this case to the Thai Department of Disease Control (DDC) in compliance with the Communicable Diseases Act. Also, we contacted the Thai DDC, which then, in collaboration with the Department of Livestock Development, was able to gather more data about the patient's goats. We found that more than 50 % of the goat samples were positive for the Rose Bengal test (RBT), a slide-type agglutination assay for detecting antibodies to *Brucella* spp. Moreover, the vaginal swabs from all female goats were positive for *C. burnetii* by conventional PCR, revealing a 5/62 (8 %) infection rate. These findings further supported that the patient was co-infected with *B. melitensis* and *C. burnetii*.

If such investigations were not done, the cases of Q fever and brucellosis would be underreported, especially among patients exposed to potentially infected animals. Therefore, advanced molecular techniques such as *C. burnetii* PCR is highly beneficial to detect DNA from a direct specimen collected from the patients with chronic Q fever as seen in this case; aside from that, the PCR test can detect acute Q fever [10]. This is crucial because the serology method cannot detect antibody responses 7–15 days after the onset of symptoms, and also there is no previous data of antibodies titer among frequent exposure people. Therefore, the PCR's ability to detect both acute and chronic Q fever in endemic area with high prevalence of potentially infected animals exposure is extremely important. This modern approach does not need the use of time-consuming paired serology and can ensure prompt diagnosis of the disease as well as provide accurate results. Consequently, it can improve the diagnostic efficiency and help patients receive early appropriate care and treatment.

4. Conclusion

Both brucellosis and Q fever are zoonotic infectious diseases that share the same host range, transmission route and clinical manifestations. Although it is rare for patients to be infected with *B. melitensis* and *C. burnetii* simultaneously, the possibility should not be ignored. Advanced molecular techniques such as *C. burnetii* PCR is highly beneficial in detecting the DNA from direct specimens collected from cases with chronic Q fever, and also allow for early detection of acute Q fever. The PCR test provides a precise diagnosis of the disease. This ultimately leads to improvements in the diagnostic process and enables early treatment, especially in regions with high prevalence of the disease and areas with high risk exposure to infected animals.

Ethical declaration and informed consent

This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB No. 0620/66 COE No. 052/2023). Written informed consent was obtained from the patient to publish all of the images, clinical data, and other data.

Data availability statement

The authors did not deposit the clinical research data in a public repository to maintain the patient's anonymity. However, the data may be available upon reasonable request to the corresponding author. And the sequencing of our patient's *C. burnetii* has already been submitted to the GenBank^{R} database (accession number. PP336779).

CRediT authorship contribution statement

Onjira Mangkalamanee: Writing – original draft, Data curation. **Suwatchareeporn Rotcheewaphan:** Writing – review & editing. **Pawat Phuensan:** Writing – review & editing. **Teerada Ponpinit:** Writing – review & editing, Resources. **Thiravat Hemachudha:** Resources. **Pattama Torvorapanit:** Writing – review & editing, Supervision, Project administration.

Declaration of generative AI and AI-assisted technologies in the writing process

During the writing process, ChatGPT was used to grammatically improve the quality of the manuscript. After using this tool, the authors reviewed and edited the content as needed. The authors take full responsibility for the content of the publication.

Declaration of competing interest

Pattama Torvorapanit reports a relationship with Health System Research Institute that includes: funding grants for other project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29685.

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