

Molecular detection of *Coxiella burnetii* infection in patients with a negative infective endocarditis culture following cardiovascular surgery

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Abstract. The present study detected *Coxiella burnetii* (*C. burnetii*) infection in patients with endocarditis with a negative bacterial culture from January, 2022 to February, 2023. For this purpose, 312 patients with endocarditis who were diagnosed and operated were included in the study after obtaining the consent of each study subject and approval from the hospital ethics committee. Following surgery, the blood samples from the patients were cultured to identify bacteria using an automatic system. In 52 cases for which the result of this culture was negative, PCR was also performed using *C. burnetii* primer pairs. As shown by the results, from the 52 blood samples from patients with endocarditis who were found to be negative for bacteria, 13 samples were found to be positive for *C. burnetii* by PCR. Among these 13 patients, all patients with a fever lasting 14 days after surgery, 6 patients with emaciation and pneumonia, and 2 patients with multi-organ failure, who then succumbed.

Introduction

Coxiella burnetii (*C. burnetii*) is an obligate intracellular bacterium and its common intermediate hosts are cattle, sheep and goats. Pathogens transmitted by the inhalation of biological product particles can travel a great distance (several kilometers), in many cases, without the patient being in direct contact with the pathogen (1). The most common infection caused by *C. burnetii* is acute Q fever, characterized by pneumonia and hepatitis. In some countries, outbreaks of >3,000 simultaneous Q fever cases have been recorded, forming an

outbreak with a mortality rate of up to 2% (France in 2007 and The Netherlands in 2010) (2). El-Mahallawy *et al* (3) evaluated the prevalence of *C. burnetii* infection in a group of 180 healthy individuals in China and found the prevalence of *C. burnetii* to be 25%. The results of their study revealed that *C. burnetii* infection was a relatively common disease in that country, in both urban and rural areas, similar to other European countries (3). Chronic Q fever due to *C. burnetii* infection usually accounts for 1-5% of *C. burnetii* infections (4). *C. burnetii* has a long incubation period; the time recorded between first exposure and clinical manifestations can vary from 1 year to >1 decade (5). Common risk factors in patients with *C. burnetii* endocarditis are the male sex (75%), an age between 40 and 70 years, valvular disease (91%); particularly the presence of prosthetic valves (30-55%) and immunocompromised patients (32%) (5).

The manifestations of infective endocarditis due to *C. burnetii* are non-specific and this is the cause of the untimely diagnosis of this condition. Almost 50% of patients with *C. burnetii* endocarditis have symptoms of acute heart failure and the majority of patients have a fever (70%), and suffer from weight loss, fatigue and anorexia (50%). Other manifestations include a rash on the extremities and mucous membranes, changes in the levels of hematological parameters, splenomegaly and renal injury caused by immune disorders (2). It has been demonstrated that some cases of endocarditis have negative blood cultures, as demonstrated in the study by Houpijian and Raoult in 2005 (6). Negative blood cultures can be caused by a variety of factors, including the method used to obtain the specimen, the culture medium used and previous antibiotic therapy (7). The study performed in France by Fournier *et al* (8) developed a multimodal strategy for the diagnosis of negative endocarditis when blood cultures were negative. The methods used in their study included classical serology and PCR analysis of the blood samples; PCR revealed an increase in diagnostic efficiency of up to 24.3%, and the authors of that study thus suggested that these tests should be used as a standard in studies on *C. Burnetii* (8).

At the Cardiovascular Surgery Unit, Bach Mai Hospital (Hanoi, Vietnam), the authors also found that among the patients undergoing cardiovascular surgery due to endocarditis,

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there were some patients with post-operative infectious complications, including some patients who had a negative blood culture. Given the complications of endocarditis caused by *C. burnetii* that have been previously reported, the present study was performed in an aim to recommend further tests and treatment regimens for patients with endocarditis (7).

Patients and methods

Patients. A total of 312 patients with endocarditis operated at the Cardiovascular Surgery Unit of Bach Mai Hospital, from January, 2022 to February, 2023, aged 17 to 74 years, male:female ratio was 1.6:1 (193 male patients), were diagnosed with endocarditis and required surgery. The patients were subjected to a full range of examinations, such as hematological analysis, coagulation analysis, microbiology tests, including hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV, as well as medical history, if necessary. Following surgery, the heart valve tissue of the patients was cultured. In the case that the results of the culture are negative, the DNA was separated from the blood, and PCR analysis for *C. burnetii* bacteria was performed using specific primer pairs. All the aforementioned procedures were approved by the Medical Ethics Committee of Bach Mai Hospital and following the written consent of the patients or their parents (for 2 patients who were underage).

PCR technique and PCR cycle. Blood DNA was separated using the Qiagen kit (Qiagen, Inc.). The primer sequences specific to *C. burnetii* used were as follows: Forward, 5'-ACG GGTGAGTAATGCGTAGG-3' and reverse, 5'-CAGTATCGG GTGCAATTCCAG-3.

The PCR assays were performed using an Eppendorf model 5382 Thermo Mixer C thermal cycler (BCE Vietnam) according to the following procedure: An initial denaturation at 95°C for 15 min; 45 cycles at 95°C for 30 sec, 57°C (for the first primer pair) or 62°C (for the second primer pair) for 30 sec and 72°C for 30 sec; and a final elongation step at 72°C for 7 min. The amplification of 5 µl DNA was performed in a total volume of 25 µl containing 10X PCR buffer (Qiagen, Inc.), 2.5 mM MgCl₂, 0.25 mM deoxynucleotide triphosphate, 25 pmol of each primer, and 1 unit of Taq DNA polymerase (Qiagen, Inc.). Agarose gel electrophoresis (2%) in the presence of ethidium bromide was used to separate the PCR products.

Hematoxylin and eosin (H&E) staining. For H&E staining, a bone marrow biopsy was performed at the posterior superior iliac spine. The sample was 10-20 mm in length and was fixed with 5% formaldehyde, and subjected to decalcification and decontamination with alcohol, xylene and molded with paraffin melting at 61°C. Staining was performed using H&E (Diapath S.P.A.) at room temperature. The thickness of sections was 0.2 mm. The sample was examined using a light microscope (Olympus Corporation) with a 40X objective.

Patient treatment. Patients with positive results for *C. burnetii* infection were treated with a regimen of doxycilin 600 mg/day for 7-10 days in combination with other antibiotics, such as imipenem and cilastatin at a dose of 1-2 g/day and their clinical progress was monitored, with periodical follow-up following hospital discharge.

Results

Out of the total of 312 patients with infective endocarditis who underwent surgery, 52 patients had negative blood and cardiac tissue cultures following surgery. Using the PCR technique with a 16s RNA primer pair of *C. burnetii* to analyze the 52 negative bacterial culture samples, 13 samples tested positive for *C. burnetii* at 460 bp; these patients had both mitral and tricuspid valve lesions, abscesses and circuit occlusion (Table I).

Patients with positive results for *C. burnetii* were tested for other viruses, including HIV, HCV, HBV, Epstein-Barr virus, cytomegalovirus and influenza, all of which yielded negative results. Following surgery, the group of patients positive for *C. burnetii* also had more severe clinical manifestations than the group of patients with negative results. The clinical lesions of the patients with *C. burnetii* infection following surgery encountered included a high fever >38°C, pneumonia, weight loss, liver failure, kidney failure, including 2 patients with severe multi-organ failure. All patients with *C. burnetii* infection had a high fever >38°C, lasting for >14 days; the longest fever duration observed was almost 40 days. Pneumonia and liver damage were recorded at a high rate in this group of patients at a rate of 84.6 and 76.9%, respectively (Table II).

The mean duration of hospitalization in the *C. burnetii*-positive group was 41.5 days, which was a markedly longer post-operative hospitalization period than the patients with negative *C. burnetii* results (Table III).

All patients infected with *C. burnetii* in the present study had anemia and thrombocytopenia; 3/13 patients had leukopenia. The average hemoglobin level of the patients was 93.6 g/l (range, 74-110 g/l). 9 patients had mild anemia, and 1 patient had moderate anemia. At its lowest, the level of hemoglobin was 74 g/l. A total of 3 patients had moderate or slightly elevated white blood cell counts, with 3/13 cases having decreased white blood cell counts, with a decreased neutrophil ratio (average, <35%). The average platelet count was 85.9x10⁹/l (Table IV).

In particular, there was 1 case (patient no. 6; Table IV) with pancytopenia and neutrophils were reduced by 0.4x10⁹/l. This patient subsequently had a bone marrow biopsy and was found to have multiple fibrin-ring granulomas (Fig. 1).

Among these cases, there were patients with both mitral and tricuspid valve lesions, or both mitral valve lesions and occlusion (Fig. 2). This patient was admitted to the hospital with breathing difficulties, which gradually increased for ~1 year. This patient was a male at 26 years of age. The tests to identify the cause of endocarditis prior to surgery were all negative. This is also one of the 2 cases of post-operative endocarditis with multi-organ failure.

Another patient succumbed 6 months following mitral valve surgery due to continued damage to the tricuspid valve, sepsis and multi-organ failure, who also tested positive for *C. burnetii*.

Discussion

There were 52/312 patients with negative results for bacterial culture following cardiovascular surgery, determined using an automatic identification system. When analyzing these 52

Table I. Results of the analysis for *Coxiella burnetii* using PCR.

Underlying etiology	PCR-positive result (no. of patients)	PCR-negative result (no. of patients)
Mitral valve	7	17
Tricuspid valve	6	12
Circuit occlusion	3	5
Abscess	3	5

Table II. Clinical manifestations following surgery in patients positive for *Coxiella burnetii* infection.

Clinical manifestations	No. of patients	%
Fever (lasting for >14 days)	13	100
Weight loss	6	46.1
Liver failure (elevation in AST/ALT levels)	11	84.6
Impaired kidney function	8	61.5
Pneumonia	10	76.9

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table III. Average duration of hospitalization.

<i>Coxiella burnetii</i> infection status	Average no. of days
Endocarditis <i>Coxiella burnetii</i> -negative (PCR)	16
Endocarditis <i>Coxiella burnetii</i> -positive (PCR)	41.5

samples, it was found that 13/52 cases had the presence of *C. burnetii* bacteria in the analyzed blood samples (Table I). Blood cultures or tissue fragments following surgery are often negative, which has been explained by a number of factors that limit blood culture results, including pre-operative antibiotic use, the specimen collection method and culture medium used, as well as previous antibiotic therapy (7). In the study by Fournier *et al* (8), it was found that the PCR method increased 24.3% sensitivity to detect the presence of *C. burnetii* in the blood of patients. *C. burnetii* is also the most commonly reported organism in cases of culture-negative endocarditis. When studying 283 cases of endocarditis with negative blood cultures, Fournier *et al* (8) found that *C. burnetii* was present in 27 cases (9.5%), exhibiting a higher proportion than other cases. Other pathogens included *Bartonella* spp., *Brucella* spp., *Tropheryma whipplei*, *Mycoplasma* spp. and *Legionella* spp., accounting for up to 5% of all diagnoses of infective endocarditis (8). According to another study by Houpiikian and Raoult (6) in a large study on culture-negative endocarditis from 1983-2001 in France, it was found that *C. burnetii* accounted for 48% of all cases diagnosed with infective endocarditis with negative blood cultures. In the present study, 13/52 cases of endocarditis with negative blood cultures were detected with *C. burnetii* infection, accounting for 25%, similar to the results of the study by Fournier *et al* (8), but markedly lower than the research results of Houpiikian and

Table IV. Changes in the levels of hematological parameters in patients positive for *Coxiella burnetii* infection.

Patient no.	Hemoglobin (g/l)	WBC (x10 ⁹ /l)	PLT (x10 ⁹ /l)
1	92	6.71	121
2	94	4.32	102
3	101	8.93	68
4	102	2.78	93
5	83	3.90	67
6	74	1.62	45
7	87	16.45	101
8	95	10.81	82
9	96	12.18	38
10	103	9.87	66
11	110	13.62	83
12	89	1.96	76
13	91	4.09	92

Raoult (6). A few case reports of post-operative complications due to *C. burnetii* have been reported by cardiovascular surgeons, such as that of Deyell *et al* in 2003 (9). In the present study, there was 1 patient who, after the first surgery to repair the mitral valve lesions and remove the wart, had to have a second surgery to correct the tricuspid valve; this patient then exhibited signs of a continuous high fever, multi-organ failure and a severe clinical course.

In a previous study, patients with valvular heart disease and Q fever due to acute *C. burnetii* infection were shown to have a 38.7% chance of developing endocarditis (10). Patient exposure to animals has been reported in 70% of cases, with patients not even realizing they have been infected (10). Manifestations of

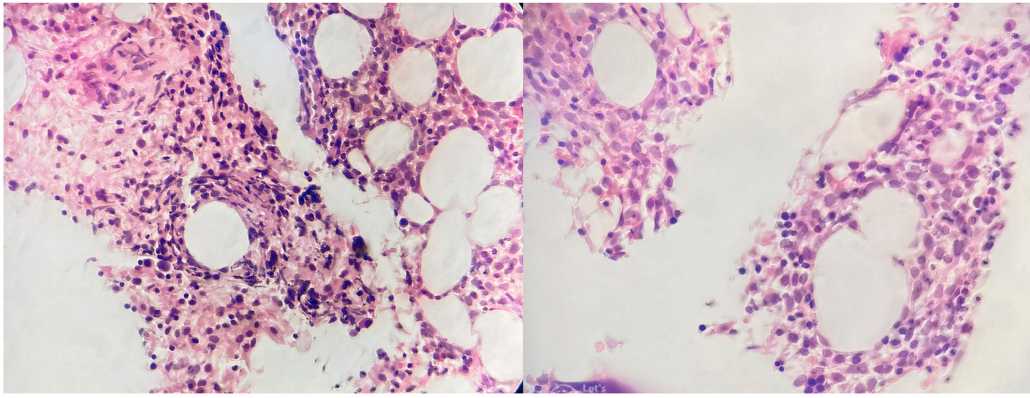


Figure 1. Bone marrow biopsy of the patient with culture-negative endocarditis. Images of fibrin-ring granuloma in the bone marrow biopsy sample of *C. burnetii*-positive patients are shown. The images on the left and right panels are from the same patient from two different fields of view. Magnification, x400.

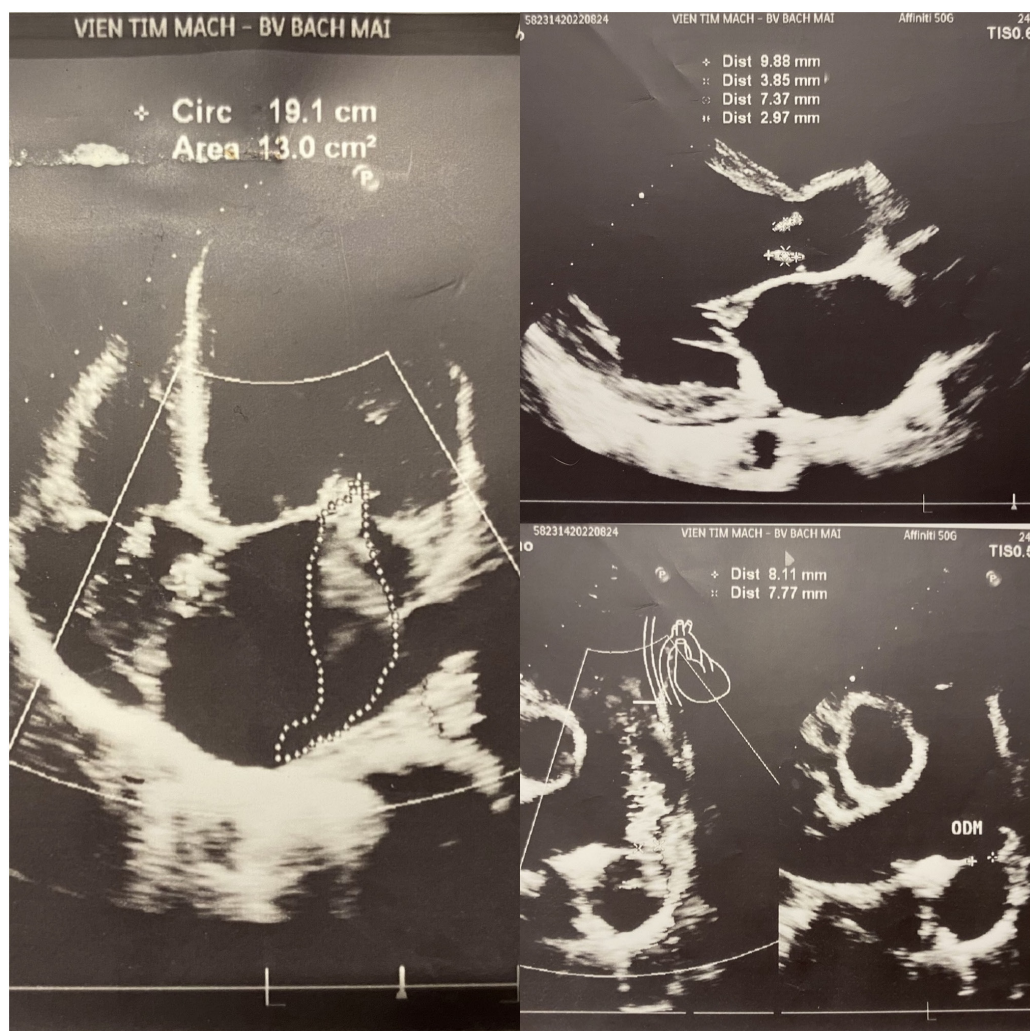


Figure 2. Doppler echocardiography of the patient with culture-negative endocarditis. There were both mitral and tricuspid valve lesions in doppler echocardiography of the patient with culture-negative endocarditis. The depicted images are from the same patient. The echocardiogram revealed severe aortic regurgitation, aortic valve vegetations, severe mitral valve regurgitation due to dilated valve annulus, moderate tricuspid valve regurgitation, remaining ductus arteriosus (6 mm in length) and a pulmonary artery diameter of 6.4 mm. Small vegetations were also observed in the ductus arteriosus and pulmonary artery.

C. burnetii causing infective endocarditis are non-specific and this is the cause of the untimely diagnosis of this condition. Almost 50% of patients have symptoms of acute heart failure and the majority of patients suffer from fever (70%), weight

loss, fatigue and anorexia (50%). Manifestations include a rash on the extremities and mucous membranes, changes in the levels of hematological parameters, splenomegaly and kidney injury caused by immune disorders (11), all of which can lead

the patients' conditions being confused with other clinical conditions.

Molecular techniques for diagnosing endocarditis from surgical tissues have been available for >20 years and have become increasingly critical in the diagnosis of endocarditis (12). These techniques detect the causative organism in the majority of cases of blood culture-negative endocarditis and may represent a major step forward in the management of endocarditis cases in which antibiotics are used before culture, in patients with inconclusive serological results, in cases where culture and serology are negative or, where serological testing is not available (13,14). Furthermore, molecular sequencing improves the understanding of the true etiology of endocarditis in different countries and represents a major step forward in the diagnostic and management of this disease (14,15).

Asian countries near Vietnam, such as China and Korea have all recorded the presence of *C. burnetii*; the study by Huang *et al* (16) recorded an outbreak of a *C. burnetii* infection in a city in China (16). The study by Bae *et al* (17) conducted in Korea, recorded 8/40 cases of *C. burnetii* negative blood culture endocarditis using PCR analysis (17).

The aforementioned studies exhibit a common factor, namely that the detection of *C. burnetii* endocarditis is difficult using conventional bacterial culture alone, and the PCR technique is considered a superior technique in determining the presence of bacteria *C. burnetii* (13,14). In the present study, the patients in the *C. burnetii*-positive group identified using the PCR method exhibited worse clinical signs than the negative group, such as a persistent high fever following surgery, pneumonia, elevated levels of liver enzymes (aspartate aminotransferase/alanine aminotransferase) and weight loss, leading to a longer hospitalization period (Table II). Deyell *et al* (9) reported a case with complications requiring re-valve surgery due to latent damage by *C. burnetii* infection. In the present study, the majority of the patients had no/unrecorded cardiac damage that warranted re-surgery. However, there was 1 case of endocarditis with damage to both the mitral and tricuspid valves (Fig. 2); following surgery, the patient exhibited a severe clinical presentation, multi-organ failure, and a 16 kg weight loss within 20 days; this patient was found to be positive for *C. burnetii* using PCR, and the patient was actively treated immediately after the infection was detected and was discharged after 62 days of treatment. This was the case with the longest hospitalization period. Multinucleated giant cells without a fibrin ring have also been described in the study by Jang *et al* (18) on *C. burnetii* infection in patients with endocarditis who had undergone surgery. Another patient in the present study died 6 months following mitral valve surgery due to continued damage to the tricuspid valve, sepsis and multi-organ failure, who also tested positive for *C. burnetii* infection. The delayed detection of the presence of *C. burnetii* may reduce the treatment efficacy. In the present study, patients positive for *C. burnetii* infection had a longer hospitalization period than the negative group (Table III). This also becomes a burden for patients and their families, doctors and as hospitals. To the best of our knowledge, the present study is the first in Vietnam using molecular biology to detect *C. burnetii* in the blood of patients with negative culture results following surgery for endocarditis. Better research results can

be achieved using 16S RNA primers analyzed on surgically operated valvular tissue.

In conclusion, endocarditis caused by *C. burnetii* infection is difficult to detect, and can cause a number of cardiovascular complications, limiting the effectiveness of cardiovascular surgery. The sources of *C. burnetii* infection are diverse, and are derived from numerous hosts; in the event that this type of infection is suspected, it is necessary to send samples to reputable laboratories for identification, in order to provide an effective intervention and treatment regimen for affected patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HDD conceived the study and was the main surgeon for the patients. ATVD and TMV obtained the patient samples and wrote the manuscript. HTVB and ATVD performed the analysis of the samples and PCR analysis. All authors have edited and agree to the reported content of the manuscript and all authors have read and approved the final manuscript. HDD and ATVD confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study received ethical approval from the Medical Ethics Committee of Bach Mai Hospital (Hanoi, Vietnam) and informed consent was obtained from all patients participating in the study. For 2 patients who were underage, the parents provided the consent.

Patient consent for publication

The patient whose Doppler echocardiography image is presented in Fig. 2 provide written informed consent for the publication of his data and the related images.

Competing interests

The authors declare that they have no competing interests.

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