Molecular detection of Coxiella burnetii infection in patients with infectious endocarditis culture negative after cardiovascular surgery in Vietnam

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Research Article

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Abstract

**Objective:** To detect Coxiella burnetii in endocarditis patients cultured negative for bacteria in a short-term study from January 2022 to February 2023 at Cardiovascular Surgery Unit, Bach Mai Hospital.

**Materials and methods:** 312 Patients with endocarditis diagnosed and operated at the Department of Cardiovascular Surgery - Bach Mai Hospital were included in the study after obtaining the consent of each study subject and the consent of the participants. hospital ethics committee. After surgery, the patient's blood sample was cultured to identify bacteria by an automatic system at the Department of Microbiology of Bach Mai hospital, 52 case was the result is negative, it will continue to be PCR analyzed with Coxiella burnetii primer pair at the Department of Microbiology, University of Natural Sciences, Vietnam National University, Hanoi.

**Results:** In a short study with 52 blood samples from endocarditis patients who were found to be negative for bacteria, 13 samples were positive for Coxiella by PCR. Among 9 patients, there were 6 patients with fever lasting 14 days after surgery, 6 patients with emaciation, pneumonia, 2 patient with multi-organ failure, emaciation.

Highlights

- Endocarditis by Coxiella burnetii is difficult to be detected by bacterial culture
- Coxiella burnetii is the most common pathogen in culture-negative endocarditis
- PCR technique is an efficient method in diagnosis of Coxiella burnetii endocarditis
- Coxiella burnetii endocarditis can cause many cardiovascular complications
- Coxiella endocarditis group has some worse postoperative complications than others

Introduction

Coxiella burnetii is an obligate intracellular bacterium and its common intermediate hosts are cattle, sheep, and goats. Pathogens transmitted by inhalation of biological product particles can travel many 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 more than 3,000 simultaneous Q fever cases have been recorded, forming an outbreak with a mortality rate of up to 2% (France 2007 and the Netherlands 2010) [2]. Parreira assessed the prevalence of C. burnetii in a group of 150 healthy blood donors in Portugal and found the prevalence of C. burnetii to be 28.7%. The results show that C. burnetii infection is a relatively common disease in this country, in both urban and rural areas, similar to other European countries [3]. Chronic Q fever due to C. burnetii usually accounts for 1–5% of C. buretii infections [4]. C. burnetii has a long incubation period, the time recorded between first exposure and clinical manifestations can vary from one year to more than a decade [5]. Common risk factors in patients with C. burnetii endocarditis are male
Manifestations of infectious endocarditis due to C. burnetii are nonspecific and this is the cause of untimely diagnosis. Nearly 50% of patients with C. burnetii endocarditis have symptoms of acute heart failure and most patients have fever (70%), weight loss, fatigue, and anorexia (50%). Other manifestations include rash on extremities and mucous membranes, changes in hematological parameters, splenomegaly, and renal injury caused by immune disorders [2]. Many studies on endocarditis have negative blood cultures, such as the study of Houpikian P et al in 2005 [6]. Negative blood cultures can be caused by a variety of factors including the way the specimen is obtained, the culture medium, and other factors [7]. A study in France by Fournier et al., developed a multimodal strategy for the diagnosis of negative endocarditis when blood cultures were negative. Methods include classical serology and PCR of blood samples, where PCR showed an increase in diagnostic efficiency of up to 24.3%, and the authors suggest that these tests should be used as standard in studies of Coxiella [8].

At the Cardiovascular Surgery Unit, Bach Mai Hospital, we also found that among the patients undergoing cardiovascular surgery due to endocarditis, there were some patients with postoperative infectious complications, including some the patient had a negative blood culture. Given the complications of endocarditis caused by Coxiella burnetii that have been reported previously by many investigators, we performed this short study to recommend further tests and treatment regimens for patients.

**Materials and methods**

**Procedure:**

A total of 312 endocarditis patients operated at the Cardiovascular Surgery Unit of Bach Mai Hospital, from January 2022 to February 2023, aged 17 to 74, were diagnosed with endocarditis and required surgery, the patient was fully tested such as: hematology, coagulation, microbiology including hepatitis B virus, hepatitis C virus and HIV as well as previous medical treatment, if necessary. After surgery, the patient is cultured heart valve tissue, if the culture results are negative, the DNA will be separated from the blood, PCR analysis for Coxiella burnetii bacteria with specific primer pairs. All the above procedures have been approved by the Medical Ethics Committee of Bach Mai Hospital (attached to the consent form) and the consent of the patient.

**PCR technique**

Blood DNA was separated by Qiagen kit. The primer sequences specific to C. burnetii we used are forward (5’-ACGGGTGAGTAATGCGTAGG-3’) and reverse (5’-CAGTATCGGGTGCAATTCCCAG-3’)

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

**Treatment**

Patients with positive C. burnetii results will be treated with a regimen of Doxycilin 600 mg/day for 7–10 days in combination with other antibiotics such as imipenem, cilastatin dose of 1–2 g/day and monitored for progress clinical, periodical follow-up after hospital discharge.

**Results**

Out of a total of 312 patients with infective endocarditis who underwent surgery, 52 patients had negative blood and cardiac tissue cultures after surgery. Of the 52 patients with negative blood cultures, 13 had positive results (Table 1).

<table>
<thead>
<tr>
<th>Underlying etiology</th>
<th>PCR positive</th>
<th>PCR negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral valve</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Tricuspid valve</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Circuit occlusion</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Abscess</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

The clinical lesions of patients with Coxiella burnetii after surgery we encountered included high fever over 38°C, pneumonia, weight loss, liver failure, kidney failure, including 1 patient with severe multi-organ failure (Table 2).
Table 2
Clinical manifestations after surgery: C. burnetii-positive

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (lasting more than 14 days)</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td>Skinny</td>
<td>6</td>
<td>46.1</td>
</tr>
<tr>
<td>Liver failure (AST/ALT elevation)</td>
<td>6</td>
<td>46.1</td>
</tr>
<tr>
<td>Impaired kidney function</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7</td>
<td>53.8</td>
</tr>
</tbody>
</table>

Among these, there are patients with both mitral and tricuspid valve lesions, or both mitral valve lesions and occlusion (Fig. 1.).

Another patient died 6 months after mitral valve surgery due to continued damage to the tricuspid valve, sepsis, and multi-organ failure also tested positive for Coxiella Burnetii.

Coxiella burnetii-positive patients had a much longer postoperative hospital stay than patients with negative Coxiella burnetii results (Table 3).

Table 3
Average length of stay

<table>
<thead>
<tr>
<th>Average number of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocarditis C. burnetii negative – PCR</td>
</tr>
<tr>
<td>endocarditis C. burnetii positive – PCR</td>
</tr>
</tbody>
</table>

Discussion

There were 52/312 patients after cardiovascular surgery with negative results for bacterial culture by automatic identification system. Analyzing the above 52 samples, we found that 13/52 cases had the presence of C. burnetii bacteria in the analyzed blood samples. Blood cultures or tissue fragments after surgery are often negative, which has been explained by many factors that limit blood culture results, including preoperative antibiotic use, specimen collection method, and culture medium and other factors [7]. In the study of Fourmier PE and colleagues, it was found that the PCR method increased 24.3% sensitivity to detect the presence of Coxiella burnetii in the blood of patients [8]. Coxiella burnetii is also the most commonly reported organism in cases of culture-negative endocarditis. When studying 283 cases of endocarditis with negative blood cultures, Fourmier et al. found that Coxiella burnetii was detected in 27 cases (9.5%), causing a higher proportion than other cases. Other pathogens include Bartonella spp., Brucella spp., Tropheryma whippelii, Mycoplasma spp. and Legionella spp., and account
for up to 5% of all diagnoses of infective endocarditis [8]. According to another study by Houpikian P et al., in a large study of 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 [7]. In our study, 13/52 cases of endocarditis with negative blood cultures were detected with Coxiella burnetii, accounting for 25%, similar to the results of Fournier et al., but much lower than the research results of Houpikian P et al. A few case reports of postoperative complications due to Coxiella burnetii have been reported by cardiovascular surgeons such as that of Deyell MW et al 2003 [9]. There was 1 patient after the first surgery to repair the mitral valve lesions, remove the wart, and also had to have a second surgery to fix the tricuspid valve, this patient then had signs of continuous high fever, multi-organ failure, severe course.

In a recent series of studies, patients with valvular heart disease and Q fever due to acute Coxiella burnetii had 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 C. burnetii causing infective endocarditis are nonspecific and this is the cause of untimely diagnosis. Nearly 50% of patients had symptoms of acute heart failure and most patients had fever (70%), weight loss, fatigue, and anorexia (50%). Manifestations include rash on extremities and mucous membranes, changes in hematologic parameters, splenomegaly, kidney injury caused by immune disorders [11], all of which make the patient sick easily confused with other clinical conditions.

Molecular techniques for diagnosing endocarditis from surgical tissue have been around for more than 20 years and have become increasingly important in the diagnosis of endocarditis [12]. This technique detects the causative organism in the majority of cases of blood culture-negative endocarditis and could 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 serologic testing is not available [13, 14]. Furthermore, molecular sequencing improves 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, South Korea have all recorded the presence of Coxiella burnetii, even in the study of Huang M et al. recorded an outbreak of a Coxiella outbreak in a city [16]. The study by Bea M et al in Korea, recorded 8/40 cases of Coxiella burnetii negative blood culture endocarditis by PCR analysis [17].

In the above studies, there is one thing in common, that patients with Coxiella burnetii endocarditis are difficult to detect by conventional bacterial culture alone, and PCR technique is considered as a superior technique in determining the presence of bacteria Coxiella burnetii [13, 14]. In our study, the patients in the group with Coxiella burnetii positive by PCR method had some worse clinical signs than the negative group, such as persistent high fever after surgery, pneumonia, elevated enzymes liver (AST/ALT), weight loss leads to longer hospital stay (Table 2). Deyell MW et al. reported a case with complication requiring re-valve surgery due to latent damage of Coxiella burnetii [9]. In our study, most of the patients had
no/unrecorded cardiac damage that warranted re-surgery. However, there was one case of endocarditis with damage to both mitral and tricuspid valves (Fig. 1) and after surgery with severe clinical presentation, multi-organ failure, 16 kg weight loss within 20 days, and was found to be positive for Coxiella burnetii by PCR, the patient was actively treated right after the disease was detected and was discharged after 62 days of treatment. This is our case with the longest hospital stay. Multinucleated giant cells without a fibrin ring have also been described in the studies of Jang Yong-Rock et al. on C.Burnetii in patients with endocarditis who had undergone surgery [18]. Another patient died 6 months after mitral valve surgery due to continued damage to the tricuspid valve, sepsis, and multi-organ failure also tested positive for Coxiella Burnetii. Delayed detection of the presence of C. Burnetii may reduce the patient's ability to treat. Patients with positive C.Burnetii had a longer hospital stay than the negative group (Table 3). This also becomes a burden for patients and their families, doctors as well as hospitals. This is the first study in Vietnam using molecular biology to detect Coxiella burnetii in the blood of patients with postoperative endocarditis culture-negative. However, we can achieve better research results by using 16S RNA primers analyzed on surgically operated valvular tissue.

**Conclusion**

Endocarditis caused by Coxiella burnetii is difficult to detect, and can cause many cardiovascular complications, limiting the effectiveness of cardiovascular surgery when treating. The source of Coxiella burnetii infection is diverse, coming from many hosts, so if there are suspicious signs, it is necessary to send samples to reputable laboratories for identification, in order to have an effective intervention and treatment regimen. more effective for the patient.

**Declarations**

**Conflicts of interest**

No conflict of interest declared

**Ethics**

The study received ethical approval from the hospital ethics committee and informed consent was obtained from all patients participating in the study.

**Sources of funding**

No funding was received for the study

**Declarations**

Hung Duong Duc conceived the idea and the main surgeon. Vinh An Do Thi and Tam Vu Minh took samples and wrote a manuscript. Ha Bui thi Viet and Vinh An Do thi took the sample analysis test by PCR. All authors have read, edited, and agreed to the reported content of the study.
References


Figures
Figure 1

Doppler echocardiogram

Image of mitral and tricuspid valve lesions in C. Burnetii positive patient