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# Diagnostic usefulness of molecular detection of *Coxiella burnetii* from blood of patients with suspected acute Q fever

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**Abstract**

Diagnosis of Q fever is difficult due to the lack of distinct clinical features that distinguish it from other febrile diseases. Serologic testing is the gold standard method for diagnosing Q fever, but antibody formation may not be detectable for 2 to 3 weeks from symptom onset, limiting early diagnosis. We thus evaluated the diagnostic utility of polymerase chain reaction (PCR) to detect *Coxellia burnetii* DNA in serum from patients with suspected acute Q fever.

All adult patients with suspected acute Q fever were prospectively enrolled at a tertiary-care hospital from January 2016 through July 2018. Acute Q fever was diagnosed using clinical and laboratory criteria: fever with at least one other symptoms (myalgia, headache, pneumonia, or hepatitis) and single phase II immunoglobulin G (IgG) antibody titers ≥1:200 or immunoglobulin M (IgM) antibody titer ≥1:50 (probable), or a fourfold increase or seroconversion in phase II IgG antibody titers as measured by indirect immunofluorescence assays between paired samples (confirmed). We performed PCR targeting the transposase gene insertion element IS1111a of *C. burnetii*.

Of the 35 patients with suspected acute Q fever, 16 (46%) were diagnosed with acute Q fever including 8 probable and 8 confirmed cases; the remaining 19 (54%) were diagnosed with other febrile diseases. The proportion of males diagnosed with Q fever was higher than those diagnosed with other febrile diseases (88% vs 44%, $P = .03$), but there were no other significant differences in clinical characteristics between the 2 groups. The Q fever PCR sensitivity was 81% (95% confidence interval [CI], 54–96), specificity was 90% (95% CI, 67–99), positive predictive value was 87% (95% CI, 63–96), and negative predictive value was 85% (95% CI, 67–94).

Q fever PCR testing using blood from patients with suspected acute Q fever seems to be a rapid and useful test for early diagnosis of Q fever.

**Abbreviations:** ALT = alanine transferase, AST = aspartate transferase, C. *burnetii* = *Coxiella burnetii*, CI = confidence interval, IFA = immunofluorescence assay, IgG = immunoglobulin G, IgM = immunoglobulin M, IQR = interquartile range, PCR = polymerase chain reaction.

**Keywords:** *Coxiella burnetii*, polymerase chain reaction, Q fever

## 1. Introduction

Q fever is a worldwide zoonotic infection caused by the Gram-negative obligate intracellular bacterium *Coxiella burnetii*, which is acquired in humans through inhalation of aerosols from infected domestic livestock and consumption of unpasteurized milk.[1] Diagnosis of Q fever is difficult because clinical awareness is limited and there are no definitive features distinguishing it from other febrile diseases. Prolonged fever is the most common feature of acute Q fever, and other symptoms and signs include

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malaise, headache, fatigue, atypical pneumonia, or hepatitis.[2] Acute Q fever often presents initially as acute febrile illness of unclear etiology that, if untreated, can progress to a persistent focalized infection such as endocarditis, with a reported mortality rate of up to 20%.[3] Therefore, recognizing Q fever in an early differential diagnosis is important for selecting an appropriate treatment and monitoring strategy.

Diagnosis of Q fever using only clinical, epidemiological, and routine laboratory findings is challenging. Serologic testing, using preferred methods such as the indirect immunofluorescence assay (IFA), is the current gold standard for diagnosing Q fever.[4] However, *C. burnetii* antibodies are only detectable 2 to 3 weeks after disease onset and serologic testing requires serum from both acute and convalescent stages of infection, which are unavailable at early diagnosis. Therefore, polymerase chain reaction (PCR) detection of *C. burnetii* DNA directly from a single serum sample has recently been considered to be a confirmatory test for acute Q fever.[5] Although there are some reports of molecular detection of *C. burnetii* DNA in buffy coat or serum from patients with acute Q fever, the sensitivity of PCR testing was reported to be approximately 33.3% to 66.7%.[6–8] We thus developed an in-house PCR test for Q fever and evaluated its diagnostic performance for Q fever detection using blood from patients with suspected acute Q fever.



## 2. Materials and methods

### 2.1. Study patients

All adult patients with suspected acute Q fever were prospectively enrolled between January 2016 and July 2018. The study was conducted in a 2700-bed tertiary-care teaching hospital in Seoul, Republic of Korea. All patients with fever (≥38°C) of unknown origin with one or more symptoms or signs including rigors, headache, hepatosplenomegaly, elevated liver enzyme, or pneumonia were included. The clinical and microbiology laboratory database was used to identify etiology of fever and exclude other common causes of fever (urinary tract infection, culture positive pneumonia, and bloodstream infection). We classified patients as having other febrile diseases when alternative clinical diagnoses were obtained from their diagnostic criteria using clinical, laboratory, and image findings. We classified cases as indeterminate when the above criteria were not met, but in which acute Q fever diagnosis could not be excluded. Information regarding contact with livestock was requested from all patients. Blood was sampled from all enrolled patients. Informed written consent was obtained from the patients. This study was approved by the Institutional Review Board of Asan Medical Center (2018-9023).

### 2.2. Definition of acute Q fever

Acute Q fever was diagnosed with serologic testing using indirect IFA. Probable cases were defined by single phase II immunoglobulin G (IgG) antibody titers ≥1:200 or immunoglobulin M (IgM) antibody titers ≥1:50. Confirmed cases were defined by a fourfold increase or seroconversion in phase II IgG antibody titers between 2 separate samples obtained 2 or more weeks apart. [9]

### 2.3. Molecular methods

**2.3.1. DNA extraction.** To detect *C. burnetii*, DNA was extracted from the blood of patients with suspected acute Q fever. About 4 mL of blood was collected in EDTA tubes, centrifuged at 134 *g* for 5 minutes and the plasma was transferred to a sterile tube and kept frozen at −20°C until further use. About 200 μL of plasma was used for DNA extraction using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction with minor modifications. For lysis, AL buffer and proteinase K were added, and samples were placed in a 70°C water bath for 10 minutes. Washing steps with these buffers were done twice, and samples were eluted in 200 μL of AE buffer and stored at −20°C until use.

**2.3.2. PCR assay.** Detection of *C. burnetii* in blood by end-point PCR was performed using primers and procedures that were modified from previous reports.[9] The gene target was derived from the transposase gene insertion element IS1111a of *C. burnetii* isolate LBCE 13265 (NCBI Nr. KT 965031.1). The forward (5'-CGG GTT AAG CGT GCT CAG TAT GTA-3') and reverse (5'-TGC CAC CGC TTT TAA TTC CTC CTC-3') primers were synthesized at around 24 bp. The end-point PCR process consisted of an initial denaturation step at 95°C for 15 minutes; 45 cycles of 95°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds; and a final elongation step at 72°C for 7 minutes. Amplification of 5 μL of DNA was performed in a total volume of 25 μL containing 10X PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 0.25 mM deoxynucleotide triphosphate, 25 pmol of each primer, and 1 unit of Taq DNA polymerase (Qiagen). Gel electrophoresis was used to separate PCR products on a 2% agarose gel containing ethidium bromide, and visualized using a GelDoc System (Clinx Science Instruments, Shanghai, China).

### 2.4. Statistical analysis

Clinical and laboratory test results were compared between patients with acute Q fever and patients with other febrile disease using Student *t* test or Fisher exact test. A $P$ value $<0.05$ was considered to be statistically significant. To investigate the diagnostic value of Q fever PCR testing, we calculated sensitivity, specificity, positive predictive value, and negative predictive value. These were determined using indirect IFA as the gold standard. The computer software used to run these statistical analyses was SPSS v. 24.0 (IBM, Armonk, New York).

## 3. Results

### 3.1. Patient characteristics

A total of 70 patients with suspected acute Q fever were enrolled during the study period. Thirty-five (50%) patients with indeterminate diagnoses were excluded from the final analysis because they did not receive an alternative clinical diagnosis after negative serologic results in paired samples (n = 4) or negative single serologic results (n = 31). Of the remaining 35 patients, 16 patients were classified as having acute Q fever and 19 patients were classified having other febrile diseases. Of these 16 patients with acute Q fever, 8 were diagnosed with confirmed Q fever and 8 with probable Q fever. No evidence of bacterial infection, leptospirosis, hemorrhagic fever with renal syndrome, scrub typhus, or murine typhus was found in the 16 patients with acute Q fever. Of the remaining patients, 19 were diagnosed with other febrile diseases, including hematologic malignancy (n = 5, 26%), rheumatologic disease (n = 4, 21%), drug hypersensitivity (n = 3, 16%), and other infectious disease (n = 7, 37%). The clinical characteristics between the 16 patients with acute Q fever and 19 patients with other febrile diseases are shown in Table 1.

**Table 1**

**Baseline characteristics of patients suspected of having acute Q fever.**

| | Total (n = 35) | Q fever (n = 16) | Other febrile diseases (n = 19) | *P* |
|---|---|---|---|---|
| Age, mean y ± SD | 53 ± 14 | 50 ± 14 | 56 ± 14 | .23 |
| Male sex | 23 (66) | 14 (88) | 9 (47) | .03 |
| Exposure to zoonotic risk factors* | 6 (17) | 4 (25) | 2 (11) | .38 |
| Immunosuppressive condition† | 6 (17) | 2 (12) | 4 (21) | .67 |
| Clinical symptoms | | | | |
| Myalgia | 31 (89) | 15 (94) | 16 (84) | .61 |
| Acute fatigue | 13 (37) | 4 (25) | 9 (47) | .29 |
| Headache | 14 (40) | 9 (56) | 5 (26) | .09 |
| Pneumonia | 3 (9) | 0 (0) | 3 (16) | .23 |
| Hepatomegaly | 7 (20) | 4 (25) | 3 (16) | .68 |
| Splenomegaly | 16 (46) | 9 (56) | 7 (37) | .32 |
| Skin rash | 10 (29) | 2 (13) | 8 (42) | .07 |
| Laboratory findings | | | | |
| Abnormal WBC count¶ | 11 (31) | 5 (31) | 6 (32) | .99 |
| Low platelet count¶ | 11 (31) | 4 (25) | 7 (37) | .49 |
| Increased liver enzymes (‡AST/ALT/ALP) | 29 (83) | 15 (94) | 14 (74) | .19 |

Data are number (%) of patients, unless otherwise indicated. SD = standard deviation. *P* values <0.05 were considered to be significant.

* Patient reported contact with cattle, tick bites and visiting to livestock farm.

† Immunosuppressive condition is defined as patients with underlying disease such as malignancy, liver cirrhosis, chronic renal failure, or those receiving immunosuppressive treatment.

¶ Normal WBC count range is between 4,000 and 10,000/uL and normal platelet count range is between 1,500,000 and 3,500,000/uL.

‡ Aspartate Transferase (AST)/Alanine Transferase (ALT)/Alkaline Phosphatase (ALP). The cutoff points of AST, ALT, and ALP was determined as 40, 40, and 120 IU/L.

The proportion of males diagnosed with Q fever was significantly higher than other febrile diseases (88% vs 44%, *P* = .03), but there were no significant differences in other clinical characteristics between 2 groups. About a quarter of acute Q fever patients had a history of contact with cattle, sheep, or goats within 2 weeks of symptom onset, but the remaining 3 quarters of acute Q fever patients did not have any zoonotic risk factors. There were no patients with acute Q fever presenting with pneumonia, and most patients (n = 15, 94%) presented with acute hepatitis. The median serum aspartate transferase (AST) and serum alanine transferase (ALT) levels among patients with hepatitis were 93 IU/L (interquartile range [IQR] 51–158) and 104 IU/L (IQR 59–169), respectively. Of the 16 acute Q fever patients, 4 underwent liver biopsies revealing granuloma with or without circumferential fibrin deposition. Of the 19 patients with other febrile diseases, 14 (74%) presented elevated serum liver enzyme levels, median AST and ALT levels were 99 IU/L (IQR 67–176) and 91 IU/L (IQR 55–180), respectively. There was no significant difference in serum AST/ALT levels between the acute Q fever and other febrile disease groups.

The final diagnosis for acute Q fever including laboratory criteria for both probable and confirmed cases was established at a median of 27 (IQR 23–33) days following the onset of symptoms. Of the 16 patients with acute Q fever, 14 received adequate treatment, and the median duration of antibiotics administration was 17 (IQR 14–27) days. No patients with acute Q fever or other febrile diseases died during the study.

### 3.2. Results of Q fever PCR tests

Q fever PCR testing was performed for all patients in each group (Fig. 1). Blood samples were collected at a median of 16 (IQR 12–33) days from the onset of symptoms and at a median of 7 (IQR 1–19) days after the first hospital visit. The diagnostic performance of the Q fever PCR test compared with the Q fever serologic test using indirect IFA as the gold standard achieved a sensitivity of 81% ([13/16], 95% confidence interval [CI], 54–96), a specificity of 90% ([17/19], 95% CI, 67–99), a positive predictive value of 87% (95% CI, 63–96), and a negative predictive value of 85% (95% CI, 67–94). Of the 8 patients with confirmed Q fever, 6 revealed positive Q fever PCR results, and 7 of the 8 patients with probable Q fever revealed positive Q fever PCR results. The detailed data of the Q fever PCR results between confirmed and probable Q cases according to serostatus are shown in Figure 2. *C. burnetii* DNA was detected in plasma from 100% (2/2) of seronegative patients, 80% (4/5) of patients with isolated phase II IgM antibodies, and 78% (7/9) of patients with phase II IgM/IgG antibodies. The median number of days from symptoms onset to blood sample collection in each group was 10, 16, and 29 days, respectively.

## 4. Discussion

We investigated clinical features of acute Q fever patient in Korea and developed an in-house PCR test for early diagnosis of acute Q fever. We found that the Q fever PCR test was optimal for screening purposes, with acceptable sensitivity (81%), specificity (90%), and predictive values (87% positive predictive value and 85% negative predictive value).

In this study, more males (88%) than females were diagnosed with acute Q fever, which has frequently been reported in previous studies of Q fever.[10] This is likely due to high occupational exposure in men as well as sex hormones that could lead to a host-dependent clinical presentation of Q fever.[11] Interestingly, only one quarter of patients with acute Q fever reported zoonotic risk factors. Among 16 patients with acute Q fever, none presented with pneumonia or abnormal chest image findings, but 15 patients (94%) showed elevated liver enzymes. Previous studies have also described the low rate of pneumonia (0%–10.7%) in Korean patients with acute Q fever.[12,13] This phenomenon may be partially explained by the lack of effort to detect pathogens in mild atypical pneumonia and the low clinical suspicion for acute Q fever in Korea.[14] Another possible explanation may be the regional variations in acute Q fever as a result of unknown factors.[15,16]

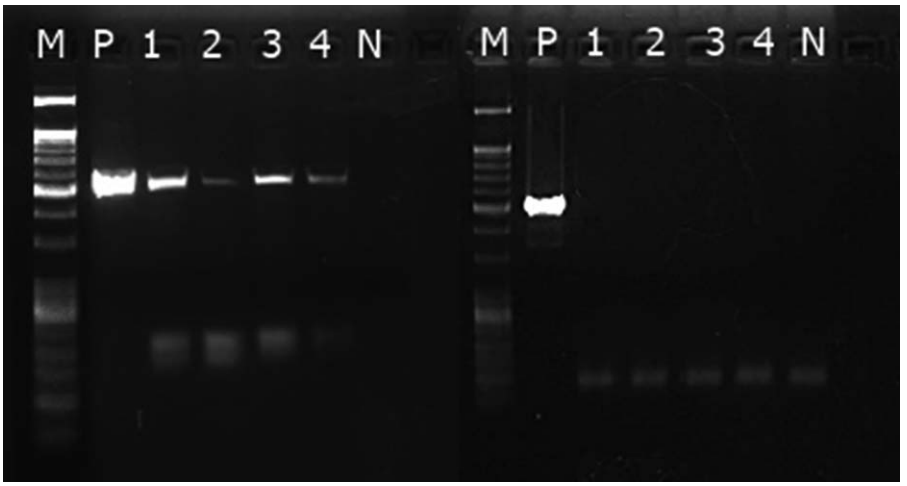


**Figure 1.** Agarose gel electrophoresis of polymerase chain reaction (PCR) products, using primers for *Coxiella burnetii* IS1111a gene. M, 50-bp DNA ladder as a size marker; P, *C. burnetii* DNA control (recombinant plasmid including 525 bp IS1111a gene of C. *burnetii*); 1–4, DNA from patients with acute Q fever (left); 1–4, DNAs from patient with other febrile disease (right); N, negative control.



Our in-house Q fever PCR detection method seems to have higher sensitivity (81%) and relatively high specificity when compared to those described in the previous studies, although wide confidence intervals prevent us from drawing a firm conclusion.[6–8] It is worth noting that the administration of doxycycline before PCR testing might have affected the results in 3 patients with false-negative results. In this study, the Q fever PCR was performed on samples collected a median of 16 days following symptom onset. It has been reported that *C. burnetii*-specific PCRs rather than antibody detection by indirect IFA are more appropriate especially when the duration of illness is ≤14 days.[5,17] In addition, Schneeberger et al reported that *C. burnetii-specific PCR results have become negative as the serological response develops.*[17] *In this study,* C. burnetii *PCR was positive in 98% seronegative sera, 90% sera with isolated phase II IgM antibodies, and 23% sera with phase II IgM/IgG antibodies. Because we used plasma instead of serum, the direct comparison between the present study and the previous studies was difficult. However, we identified a similar trend as other studies where PCR sensitivity was higher at earlier infection stages before seroconversion.*[17]

Serologic testing using indirect IFA requires 10 to 14 days to send the samples to a reference laboratory equipped with an adequate microscopy and trained technicians and to receive the

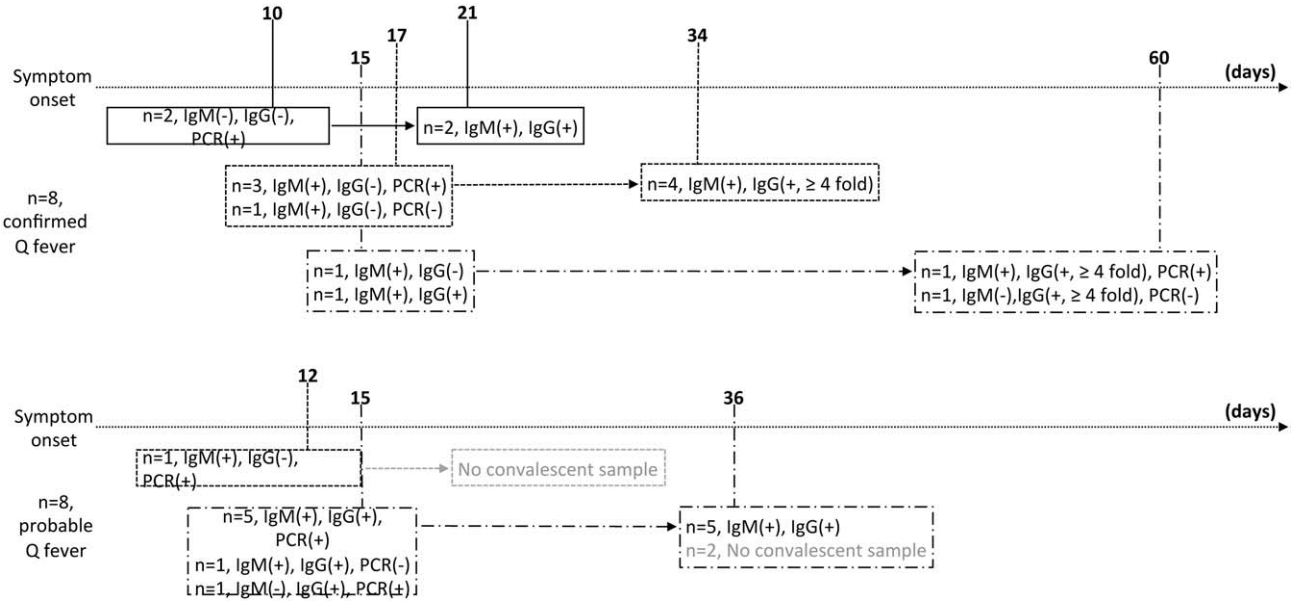


**Figure 2.** Phase II antibody distribution of the patients with confirmed and probable Q fever and Q fever polymerase chain reaction (PCR) results of each serologic group. IgM (+) = phase II immunoglobulin M (IgM) antibody titer ≥1:50; IgG (+) = phase II immunoglobulin G (IgG) antibody titer ≥1:200; PCR (+) = positive detection of *Coxiella burnetii* DNA by PCR.

results. Because of this, the final diagnosis for acute Q fever including serological criteria was usually made in real clinical practice at about 27 days from the onset of symptoms. However, using the Q fever PCR, we were able to test multiple samples at one time, and confirm the results on the same day. Therefore, we assume that PCR detection of *C. burnetii* DNA for the early diagnosis of acute Q fever is more useful than the serologic test using indirect IFA.

There are certain limitations in this study. First, we could not enroll a sufficient number of acute Q fever patients to describe the clinical features of acute Q fever in Korea. However, compared with the previous studies, this study provides a relatively large sample size to evaluate the usefulness of the Q fever PCR. Second, because this study enrolled patients who visited a tertiary-care hospital, if patients with acute Q fever presented with a flu-like illness or other mild disease, they may not have been included. Third, we initially enrolled 70 patients with suspected acute Q fever, but 35 patients (50% of the total patients) were excluded in the study due to an "intermediated diagnosis." This may have introduced selection bias that affected the outcome of our study. Fourth, because *C. burnetii* is an intracellular bacterium that multiplies in monocytes and macrophages of infected hosts, it may be possible to improve the sensitivity of Q fever PCR by extracting DNA from buffy coat rather than plasma.

In conclusion, we could improve our understanding of the clinical features of presentation of acute Q fever in Korea and develop a useful in-house PCR from the blood in patients with suspected acute Q fever. This in-house PCR test could help to distinguish Q fever from other febrile diseases more rapidly, improving differential diagnosis time.

## Author contributions

**Investigation:** Moonsuk Bae, Choong Eun Jin.
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**Writing – review and editing:** Yong Shin, Sung-Han Kim.

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