Short Communication

Use of a Single-Tube Nested Real-Time PCR Assay to Facilitate the Early Diagnosis of Acute Q Fever

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SUMMARY: We have developed a single-tube nested real-time PCR (STN-RT PCR) assay using the repetitive, transposon-like element IS6110 as the DNA target to facilitate early diagnosis of acute Q fever. The use of our proposed diagnostic procedures, including IgM detection by serology and the STN-RT PCR assay, significantly increased the diagnostic sensitivity for Q fever to 78%, compared to 29% when serology alone was used for subjects providing mainly acute-phase blood samples.

Q fever is a ubiquitous zoonosis caused by Coxiella burnetii, a small pleomorphic Gram-negative intracellular bacillus that occurs worldwide (1). The clinical manifestations of Q fever are varied, with influenza-like illness, hepatitis, or pneumonia being the most common presentations in acute infections and infective endocarditis in chronic infections. Since the clinical presentations of Q fever are usually nonspecific, a definite diagnosis mainly requires laboratory confirmation.

Serology is currently the diagnostic method of choice for Q fever, with indirect immunofluorescence assay (IFA) being the most commonly used method (1, 2). However, as antibodies may not appear until late into the course of the disease, it is often difficult to make an early diagnosis based solely on serology for patients who present early. Since early diagnosis would be helpful both for treatment of patients and to initiate a timely response in the event of a Q fever outbreak, the development of a diagnostic assay based on direct antigen detection or polymerase chain reaction (PCR) would be an invaluable supplement to serology (3, 4).

Q fever is a notifiable disease in Taiwan, and physicians are required to submit blood samples from patients to the rickettsial reference laboratory of the Taiwan Centers for Disease Control (TCDC) for confirmation. Our current diagnostic method relies on serology using a commercial IFA kit (Focus Diagnostics, Cypress, Calif., USA). The criterion for confirmation of acute Q fever is a titer of anti-phase II IgM ≥ 80 in any acute-phase serum sample, or a fourfold increase of anti-phase II IgG titers between paired acute- and convalescent-phase serum samples in patients with symptoms suggestive of Q fever. To facilitate the early diagnosis of acute Q fever, a modified PCR-based assay, namely a single-tube nested real-time PCR (STN-RT PCR) assay, has been developed in our laboratory. The results of our PCR testing and a recommended diagnostic algorithm for Q fever are presented herein.

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A total of 93 blood samples from patients with confirmed acute Q fever by IFA were tested with our PCR assay. Each patient provided only one blood sample for testing. If a patient had provided two or more blood samples, only the first was retrieved. A further 86 blood samples from subjects suspected to have Q fever but who turned out to be Q fever seronegative were selected as negative controls. The sensitivity of using either the STN-RT PCR assay alone or together with serology for the diagnosis of Q fever was compared with that of serology alone by McNemar’s test. The influence of IgM antibody in blood samples on the detection of C. burnetii by PCR assay was examined using the chi-square test.

Two major modifications were required during the development of our PCR assay. First, C. burnetii DNA was extracted directly from blood samples using the automated MagNA Pure nucleic acid isolation system (Roche Diagnostics, Almere, the Netherlands) to facilitate processing and reduce contamination with respect to the traditional RT-PCR method. This modification allowed C. burnetii DNA to be obtained easily from blood instead of the commonly used serum samples. Briefly, DNA was extracted from 400 μL of whole blood using the Total Nucleic Acid Isolation Kit-Large Volume (Roche Diagnostics, Mannheim, Germany) following the manufacturer’s instructions. The purified nucleic acid was eluted in a final volume of 100 μL. The MagNA Pure performed all steps in the procedure automatically. The resulting nucleic acids were bound to magnetic beads inside the pipette tips, washed free of impurities, and finally eluted from the magnetic beads into a cartridge. A total of 32 samples could be extracted in a single run, and this process took only around 2 h.

Second, we modified our single-tube nested PCR procedure so that it could be used as a real-time PCR assay. Two pairs of oligonucleotide primers were designed as described previously (5). Each 50-μL reaction mixture consisted of 25 μL of master mix, 2.5 μL of each of the four primers, QF9 (5′-TATGGTATCCACCACCGTACGT-3′) at a final concentration of 0.005 μM, QF10 (5′-CCCACACACCACACCTTATTCC-3′) at a final concentration of 0.005 μM, QF11 (5′-GACCGAACCACCATGATTC-3′) at a final concentration of 0.5 μM, QF12 (5′-CTTTAACAGCGCGT-GTA-3′) at a final concentration of 0.5 μM, and TaqMan probes (5′-TATGGTATCCACCACCGTACGT-3′) at a final concentration of 0.005 μM.
ACGT-3′) at a final concentration of 0.5 µM, 10 µL of sterile distilled water, and 5 µL of DNA template. The QF9 and QF10 primers, which were designed to amplify a 687-bp fragment of the IS1111 repetitive element, were used for external amplification, whereas internal amplification was performed using the QF11 and QF12 primers, which amplified a 203-bp fragment. The cycling conditions in an MX4000™ PCR machine (Stratagene, La Jolla, Calif., USA) were as follows: external amplification protocol was performed at 95°C for 10 min, followed by 5 denaturation cycles at 94°C for 30 s. The annealing temperature followed a touch-down profile from 66°C to 62°C for 30 s, which decreased by –1°C for each amplification cycle, and extension at 72°C for 60 s followed by 10 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 60 s. Internal amplification involved 40 denaturation cycles at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 60 s. Positive PCR products were sequenced externally (Mission Biotech, Gainesvill, Fla., USA). Sequence analysis was performed with NCBI BLAST using microbial genomes (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi).

Of the 93 blood samples from acute Q fever subjects, 27 (sensitivity, 29%) had serological profiles indicating acute Q fever with titers of anti-phase II IgM ≥ 80. In comparison, 56 (sensitivity, 60%) tested positive in our STN-RT PCR assay. None of the 86 control samples gave positive PCR results, thus confirming the specificity of 100%. The results of both assays for blood samples with different time intervals between illness onset and sampling date are summarized in Table 1.

Of the 27 samples with diagnostic anti-phase II IgM serology, only 10 (37%) were PCR-positive. In contrast, 46 (70%) of the remaining 66 samples without diagnostic IgM serology were PCR-positive. Since the absence of IgM antibody was associated with a positive PCR result ($P < 0.05$), and the majority of samples with negative serology were from subjects whose blood was sampled within 2 weeks of illness onset, routine PCR testing would be recommended for all subjects who present within 2 weeks after onset of illness. With a diagnostic protocol including both serology and STN-RT PCR testing, the diagnostic sensitivity of Q fever increased significantly to 78% (73/93), compared to 29% (27/93) based on serology alone ($P < 0.05$). We therefore recommend that this combined diagnostic strategy be used to facilitate the early diagnosis of acute Q fever.

Table 1. Sensitivities of different detection methods and combinations for the diagnosis of acute Q fever

<table>
<thead>
<tr>
<th>Method</th>
<th>% Sensitivity$^1$ for different time periods after illness onset</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1–6</td>
<td>Day 7–14</td>
</tr>
<tr>
<td>Titer of anti-phase II IgM ≥ 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STN-RT PCR</td>
<td>13 (6/45)</td>
<td>35 (14/40)</td>
</tr>
<tr>
<td>Titer of anti-phase II IgM ≥ 80 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STN-RT PCR</td>
<td>67 (30/45)</td>
<td>60 (24/40)</td>
</tr>
<tr>
<td></td>
<td>73 (33/45)</td>
<td>80 (32/40)</td>
</tr>
</tbody>
</table>

$^1$: Number of positive results/total number of tested samples are presented in parentheses.

$^2$: Significantly higher than that of serology alone with 29%.

Acknowledgments This work was in part supported by grants DOH96-DC-2029 from the Centers for Disease Control, Department of Health, Taiwan.

Conflict of interest None to declare.

REFERENCES