Follow-up of 686 Patients With Acute Q Fever and Detection of Chronic Infection

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Background. Recent outbreaks in the Netherlands allowed for laboratory follow-up of a large series of patients with acute Q fever and for evaluation of test algorithms to detect chronic Q fever, a condition with considerable morbidity and mortality.

Methods. For 686 patients with acute Q fever, IgG antibodies to *Coxiella burnetii* were determined using an immunofluorescence assay at 3, 6, and 12 months of follow-up. Polymerase chain reaction (PCR) was performed after 12 months and on earlier serum samples with an IgG phase I antibody titer ≥1:1024.

Results. In 43% of patients, the IgG phase II antibody titers remained high (≥1:1024) at 3, 6, and 12 months of follow-up. Three months after acute Q fever, 14% of the patients had an IgG phase I titer ≥1:1024, which became negative later in 81%. IgG phase I antibody titers were rarely higher than phase II titers. Eleven cases of chronic Q fever were identified on the basis of serological profile, PCR results, and clinical presentation. Six of these patients were known to have clinical risk factors at the time of acute Q fever. In a comparison of various serological algorithms, IgG phase I titer ≥1:1024 at 6 months had the most favorable sensitivity and positive predictive value for the detection of chronic Q fever.

Conclusions. The wide variation of serological and PCR results during the follow-up of acute Q fever implies that the diagnosis of chronic Q fever, necessitating long-term antibiotic treatment, must be based primarily on clinical grounds. Different serological follow-up strategies are needed for patients with and without known risk factors for chronic Q fever.

Q fever, a zoonotic disease caused by the bacterium *Coxiella burnetii*, emerged in the Netherlands in small ruminants in 2005 and in humans in 2007. By the end of 2009, >3500 patients had been identified, making it the largest outbreak of human Q fever recorded to date [1]. Acute Q fever may develop into chronic Q fever, a potentially lethal disease in 2% of patients [2]. Patients with previous cardiac valve pathology, aneurysms, or vascular grafts; immunocompromized persons; and pregnant women are at risk for chronic Q fever [3]. Serological follow-up of patients with acute Q fever is thought to be essential for the early detection and prompt treatment of chronic Q fever.

Infection with *C. burnetii* is accompanied by antigenic phase variation; in general, phase II antibodies are followed by phase I antibodies. IgM antibodies to phase II appear 10–17 days after the onset of acute illness, followed by the IgG antibodies to phase II. Subsequently, in chronic infection, IgG phase I antibodies predominate. An IgG phase I antibody titer ≥1:800 is considered highly predictive for chronic Q fever [4–6]. Also indicative of chronic Q fever are IgG phase I antibody levels higher than IgG phase II levels, the persistence or increase of high levels of IgG phase I antibodies, or the reappearance of such antibodies [6–8].

During acute Q fever, *C. burnetii* DNA can only be detected in serum samples with use of polymerase chain reaction (PCR) during the first 2 weeks of illness, with
reported sensitivities of 26%–98% [9–11]. In chronic Q fever, the serum PCR can again become positive, and it has been proposed that the final diagnosis of chronic Q fever can be made when a suspect serological profile is combined with a positive PCR result [12].

Immunofluorescence assay (IFA) is considered to be the reference method for serological diagnosis of acute and chronic Q fever [4]. However, there is considerable uncertainty about the value of serologic testing for identifying chronic cases. Literature on chronic Q fever is based on mostly data and serum samples that were submitted to the French National Reference Center for Rickettsial Diseases and had been collected from different areas over many years and merged for analysis [13]. The situation in the Netherlands allowed us to monitor a large number of patients with acute Q fever in an outbreak that was clearly defined in time and place. The regional Microbiology Laboratory of the Jeroen Bosch Hospital in ’s-Hertogenbosch is located centrally in an area with high incidence Q fever in the southern region of the Netherlands. It has an estimated catchment population of 550,000 and serves 2 large tertiary hospitals and ~400 general practitioners. The laboratory implemented a screening and follow-up protocol in line with the most common recommendations in the literature. This suggests serological follow-up of all acute cases at 3, 6, and 12 months after diagnosis [6, 14].

The objectives of this study were (1) to describe the serological profiles and PCR results over 1 year of follow-up for 686 patients who received a diagnosis of acute Q fever in the regional laboratory; (2) to identify the patients from this group with chronic Q fever, based on serologic findings, PCR results, and clinical presentation; and (3) to define the best serological algorithm for the detection of chronic or potentially chronic Q fever.

MATERIALS AND METHODS

Definition of Acute Q Fever and Serological Follow-up

Patients were referred by a general practitioner or a medical specialist for laboratory confirmation of the presumptive diagnosis of acute Q fever. We defined laboratory-confirmed acute Q fever as both IgM and IgG phase II antibody titres ≥1:32 with use of IFA (Focus Diagnostics) or a positive PCR result preceding seroconversion in IFA. All patients who received a diagnosis during 2007 and 2008 and had laboratory-confirmed acute Q fever were asked to provide a follow-up serum sample at 3, 6, and 12 months. This retrospective analysis only included patients who had submitted at least a serum sample at the 12-month follow-up visit. IgG phase I antibody and IgG phase II antibody titers were determined using IFA in all follow-up serum samples with use of 2-fold dilutions, initially with a dilution of 1:32.

PCR

PCR was retrospectively performed on all 12-month serum samples and on earlier serum samples with an IgG phase I antibody titer ≥1:1024, if sufficient serum was still available. The real-time PCR on serum samples, targeting the IS1111 insertion element, has been described elsewhere [11]. In short, DNA was extracted from 500 μL of serum, to which 10 μL of phocine herpes virus (PhHV), which served as an internal control, was added [15]. PCRs with PhHV cycle threshold (Ct) values above the PhHV mean Ct value of noninhibited samples + 2 standard deviations (SDs) were considered to be inhibited. In all the runs, we included DNA isolation controls (NC; a mock isolation submitted to PCR) and no-template controls (NTC; a PCR with PCR ingredients and water instead of sample) to monitor the presence of contaminants in isolation and/or PCR reagents.

Definition of Chronic Q Fever

Confirmed chronic Q fever was defined as the presence of at least 2 of the following 3 criteria: (1) IgG phase I antibody titer ≥1:1024, (2) positive PCR result ≥3 months after the acute episode, and (3) clinical or radiological signs interpreted by a medical specialist as highly suggestive of chronic Q fever. However, the medical diagnosis of chronic Q fever and the decision to treat were made independently of the study by a team of medical specialists and were based on serological and PCR test results, the presence of clinical risk factors, radiological imaging results, clinical presentation, comorbidities, and other patient characteristics. We included patients with chronic Q fever who had received a diagnosis before 1 July 2010.

Comparing Serological Algorithms

We calculated the sensitivity and the positive predictive value for different serological screening options against 2 outcome measures: (1) IgG phase I antibody titer ≥1:1024 at 12 months and (2) confirmed chronic Q fever. Sensitivity was considered to be important, because the aim of a follow-up strategy is not to miss cases of chronic Q fever. The positive predictive value was defined in this context as the probability that a patient has or will develop chronic Q fever in the event of a positive screening test result. Specificity and a negative predictive value are less useful measures, because chronic Q fever is a rare disease and the vast majority of those screened will, therefore, not have chronic Q fever. Data were analyzed using Excel (Microsoft) and SPSS, version 18.0 (SPSS).

RESULTS

Serological Follow-up Profile

Twelve-month follow-up serological test results were available for 686 patients, 37 of whom received a diagnosis of acute Q fever in 2007 and 649 in 2008. The first serum samples on which the diagnosis of acute Q fever was made were collected from 28
June 2007 through 19 December 2008. The onset of clinical signs attributed to acute Q fever occurred from 1 May 2007 through 29 November 2008 in the 536 (78%) of 686 patients for whom this information was available. At 3-month follow-up, serological test results were available for 622 (91%) of 686 patients and for 587 (86%) of 686 patients at 6 months.

Figure 1 summarizes the serological test results for the total group of 686 patients with acute Q fever. There was a wide range of IgG antibody responses. In 72 (13%) of the 561 patients who provided 3 follow-up samples, IgG phase I antibodies were not detected at any time. At 3 months, 84 (14%) of 622 patients had a high IgG phase I antibody titer ($\geq 1:1024$) that remained high in 25 of 72 patients at 6 months (no sample was available at 6 months for 12 patients) and in 16 of 84 patients at 12 months. At 6 months, an additional 21 patients had a high IgG phase I antibody titer, 7 of which were still high at 12 months, whereas 9 patients had high IgG phase I antibody titers for the first time at 12 months. In contrast, a large number of patients (241 [43%] of 561) had persistently high ($\geq 1:1024$) IgG II antibody titers at 3, 6, and 12 months of follow-up.

**PCR Result**
Sufficient serum was available for retrospective PCR analysis in 58 patients with a high IgG phase I antibody titer ($\geq 1:1024$) at 3 months of follow-up. Only 3 of these tested positive, with Ct values ranging from 34.6 to 37.3. Two of these samples, one from a patient who developed chronic Q fever, tested positive in duplicate. The third sample tested positive in 1 of the 2 duplicate PCRs. Only this last sample contained enough serum for PCR retesting; no *C. burnetii* DNA was detected. Tests on a random sample of 55 3-month serum samples from patients with an IgG phase I antibody titer <1:1024 resulted in 1 positive PCR result, with Ct values of 36.2 and 36.7. Retesting of this sample yielded a negative PCR result in duplicate. Of the 41 serum samples available from patients with an IgG phase I antibody titer $\geq 1:1024$ at 6 months of follow-up, 5 tested positive. The sample from the aforementioned patient who had a positive PCR result at 3 months and who developed chronic Q fever displayed Ct values of 34.9 and 36.9 (patient 6 in Table 1). The other 4 samples tested positive in 1 of the 2 duplicate PCRs, with Ct values ranging from 35.1 to 36.4. Only one of these samples contained enough serum for PCR retesting. This again yielded 1 positive result, with a Ct value of 37.0, and 1 negative PCR result.

A random sample of 47 serum specimens from patients with an IgG phase I titer <1:1024 at 6 months all tested negative with use of the *C. burnetii* real-time PCR. Of the 672 12-month samples tested using the PCR, 664 samples tested negative, 7 samples tested positive, and 1 sample could not be evaluated because of inhibition. The only sample that tested positive in duplicate, with Ct values of 33.9 and 34.2, was from a patient with chronic Q fever patient (patient 4 in Table 1). The other 6 were positive in 1 of the 2 duplicate PCRs, with Ct values of 36.3, 36.9, 41.7, 41.8, 41.9, and 43.7. Only 1 sample with a Ct value of 36.8 was suitable for retesting and subsequently appeared to be negative in duplicate. All real-time *C. burnetii* negative controls and nontemplate controls tested during the analyses remained negative.

**Characteristics of Patients With Chronic Q Fever**
By 1 July 2010, 11 patients (1.6%) in the group of 686 patients with acute Q fever had a diagnosis of chronic Q fever. The mean age of the patients with chronic Q fever was 66 years (range, 51–82 years), 73% of whom were male. In comparison, the patients who did not develop chronic Q fever had a mean age of 51 years (range, 6–89 years), 59% of whom were male. The difference in age was statistically significant (Student's t test, *P* < .001), but the difference in sex was not statistically significant ($\chi^2$ test, *P* = .282).

Table 1 shows details of the 11 patients who were identified with chronic Q fever. Seven of these patients were started on treatment with doxycycline (200 mg daily) and hydroxychloroquine (600 mg daily). Patient 2 in Table 1 did not have a suspect serological profile during the 12-month follow-up period but was admitted to hospital 21 months after the acute Q fever episode with a burst aneurysm and with a positive PCR result on serum and vascular tissue samples. Patient 3 initially presented with streptococcal endocarditis but received a diagnosis of chronic Q fever shortly afterward and was treated accordingly. Patient 9 had high antibody titers at initial presentation and received 6 months of prophylactic treatment with doxycycline and hydroxychloroquine.
because of a recent cardiac valve replacement. This patient showed sharply decreasing IgG I titers (Table 1). Patients 10 and 11 did not undergo additional diagnostic procedures (such as radiologic imaging), and it remains unknown whether they had cardiovascular risk factors or clinical abnormalities. Nine of the 11 patients with chronic Q fever showed persistent or increasing titers for IgG phase I in addition to IgG phase II antibody titers. The 2 patients who showed decreasing IgG phase I antibody titers to a titer, 1:1024 (patients 8 and 9 in Table 1) had been started on long-term antibiotic treatment within a few months after the acute Q fever episode. Eight (73%) of the 11 patients with confirmed chronic Q fever had IgG phase I antibody titer 1:1024 at 6 months, whereas this occurred in only 13 (2%) of 674 of the other patients. Two patients with chronic Q fever had an IgG phase I antibody titer greater than the IgG phase II antibody titer, one at 3 and 6 months and one at 6 and 12 months. There was only one other patient in the full data set who had an IgG phase I titer greater than the IgG phase II titer (at 3-month follow-up only). Six of the patients with chronic Q fever were already known to have clinical risk factors at the time of the acute Q fever diagnosis and were therefore monitored closely. Four others (patients 5, 7, 10, and 11 in Table 1) were not known to have clinical risk factors and were detected at 6-month follow-up.

Serological Algorithms

Table 2 shows the sensitivity and positive predictive values of various serological screening options at 3 and 6 months of follow-up, for the detection of chronic Q fever and for the detection of an IgG phase I antibody titer 1:1024 at 12 months. The 2 patients with chronic Q fever who were started on long-term antibiotic treatment shortly after the acute Q fever episode were not included in this analysis. The most favorable combination of sensitivity and positive predictive value was obtained by screening for an IgG phase I antibody titer 1:1024 at 6 months.

DISCUSSION

A unique series of 686 patients with acute Q fever was studied during 12 months of follow-up. Our serological results differ from data reported by others, in that high IgG phase I antibody titers at 3 months of follow-up were not predictive of chronic Q fever, and IgG phase I antibody titers greater than IgG phase II antibody titers were rarely seen [4, 6]. In our analysis, an IgG phase I antibody titer 1:1024 at 6 months had the highest sensitivity for detecting chronic Q fever, but the probability of persons with this profile actually having chronic Q fever was

Table 1. Characteristics of Confirmed Chronic Q Fever Cases Among 686 Patients With Acute Q Fever

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age</th>
<th>Serology at 3 months</th>
<th>Serology at 6 months</th>
<th>Serology at 12 months</th>
<th>PCR (Ct value)</th>
<th>Known clinical risk factor at time of diagnosis acute Q fever</th>
<th>Clinical signs at follow-up</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG I</td>
<td>IgG II</td>
<td>IgG I</td>
<td>IgG II</td>
<td>At 6 months</td>
<td>At 12 months</td>
<td>At 3 months</td>
</tr>
<tr>
<td>1</td>
<td>m</td>
<td>75</td>
<td>1:128</td>
<td>1:4096</td>
<td>1:8192</td>
<td>1:32768</td>
<td></td>
<td></td>
<td>1:2048</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>76</td>
<td>1:32</td>
<td>1:4096</td>
<td>1:128</td>
<td>1:1024</td>
<td></td>
<td></td>
<td>1:512</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>70</td>
<td>1:32768</td>
<td>1:16384</td>
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<td>1:16384</td>
<td></td>
<td></td>
<td>1:4096</td>
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<tr>
<td>4</td>
<td>m</td>
<td>54</td>
<td>1:1024</td>
<td>1:4096</td>
<td>1:1024</td>
<td>1:4096</td>
<td></td>
<td></td>
<td>1:8192</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>51</td>
<td>na</td>
<td>na</td>
<td>1:8192</td>
<td>1:32768</td>
<td></td>
<td></td>
<td>1:8192</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>63</td>
<td>1:2048</td>
<td>1:8192</td>
<td>1:1024</td>
<td>1:4096</td>
<td></td>
<td></td>
<td>1:16384</td>
</tr>
<tr>
<td>7</td>
<td>f</td>
<td>51</td>
<td>1:256</td>
<td>1:2048</td>
<td>1:8192</td>
<td>1:2048</td>
<td></td>
<td></td>
<td>1:8192</td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>66</td>
<td>1:32768</td>
<td>1:65536</td>
<td>1:4096</td>
<td>1:16384</td>
<td></td>
<td></td>
<td>1:512</td>
</tr>
<tr>
<td>9</td>
<td>f</td>
<td>61</td>
<td>1:4096</td>
<td>1:65536</td>
<td>1:256</td>
<td>1:4096</td>
<td></td>
<td></td>
<td>1:64</td>
</tr>
<tr>
<td>10</td>
<td>f</td>
<td>82</td>
<td>na</td>
<td>na</td>
<td>1:1024</td>
<td>1:4096</td>
<td></td>
<td></td>
<td>1:2048</td>
</tr>
<tr>
<td>11</td>
<td>m</td>
<td>73</td>
<td>1:256</td>
<td>1:2048</td>
<td>1:1024</td>
<td>1:4096</td>
<td></td>
<td></td>
<td>1:2048</td>
</tr>
</tbody>
</table>

NOTE. na, no serum sample available; undet, undetermined Ct value.
low. Although most elevated IgG phase I antibody titers \((\geq 1:1024)\) at 3 months after the acute Q fever episode subsequently normalized, elevated IgG phase II antibody titers at 3 months often remained high at 6 and 12 months of follow-up. This is consistent with findings from previous studies that IgG phase II antibody titers remain at a constant high level for almost a year and subsequently decrease slowly [4, 16]. A persistently high IgG phase II antibody titer has been suggested as an indicator of chronic Q fever [14]. However, this had a very low positive predictive value in our study population.

Our PCR results did not show a consistent pattern, and the Ct values of positive test results were generally high (i.e., the positive PCR signals were weak). Nine patients who were not suspected to have chronic Q fever tested positive, 2 of them in duplicate. Four of these samples could be retested, and the positive signal was confirmed in 1 sample only. Although none of the control subjects tested positive in any of the runs, we cannot exclude contamination, especially considering the number of Q fever patients with acute Q fever, it is essential to make a distinction between patients with and without known risk factors for chronic Q fever.

We conclude that for the microbiological follow-up of patients with acute Q fever, it is essential to make a distinction between patients with and without known risk factors for chronic Q fever. A stringent follow-up scheme is required if patients with acute Q fever have risk factors, as did 6 of the 11 patients with chronic cases in our study. The wide variation in serological and PCR test results at different follow-up times emphasizes the fact that the diagnosis of chronic Q fever and the decision to start long-term antibiotic treatment must be based primarily on clinical grounds. At our hospital, we perform serological and PCR follow-up testing at 3, 6, and 12 months for patients with preexisting cardiac valve disorders. In line with international recommendations, active screening for cardiac valve disorders among patients with acute Q fever was performed in the early stages of the outbreak in the Netherlands. However, in 2007, routine echocardiography revealed cardiac valve abnormalities in 59% of 66 patients with acute Q fever, the majority of whom had no clinical signs. None of the patients progressed to chronic disease, and echocardiographic screening was discontinued [23].

One limitation of this study is that we do not have extensive clinical details on the majority of patients who had a serological profile of acute Q fever. However, requests for laboratory diagnosis of Q fever were either from general practitioners or from hospital physicians because of clinical signs suggestive of acute Q fever.

We conclude that for the microbiological follow-up of patients with acute Q fever, it is essential to make a distinction between patients with and without known risk factors for chronic Q fever. A stringent follow-up scheme is required if patients with acute Q fever have risk factors, as did 6 of the 11 patients with chronic cases in our study. The wide variation in serological and PCR test results at different follow-up times emphasizes the fact that the diagnosis of chronic Q fever and the decision to start long-term antibiotic treatment must be based primarily on clinical grounds. At our hospital, we perform serological and PCR follow-up testing at 3, 6, and 12 months for patients with risk factors. In this group, persistently high IgG antibody titers against both phase I and II indicate chronic Q fever. Three-month follow-up screening is not useful for patients with acute Q fever without risk factors. On the basis of the experience gained, our hospital now performs tests at 9 months only after an acute episode of Q fever. We are uncertain as to the

### Table 2. Sensitivity and Positive Predictive Value (PPV) of High Titers of IgG Antibodies to *Coxiella burnetii* Phase I and Phase II Antigens in Immunofluorescence Assay at 3 and 6 Months After an Acute Q Fever Episode for Detection of Chronic Q Fever and for Detection of a High IgG Phase I Antibody Level at 12-month Follow-up

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Follow-up (months)</th>
<th>IgG I (\geq 1:1024) at 12 months (n=34)</th>
<th>Confirmed chronic Q fever (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>Sensitivity (95% CI)</td>
<td>PPV (95% CI)</td>
</tr>
<tr>
<td>IgG I (\geq 1:1024)</td>
<td>3</td>
<td>622</td>
<td>50% (32–68)</td>
</tr>
<tr>
<td>IgG I (\geq 1:4096)</td>
<td>3</td>
<td>622</td>
<td>9% (2–26)</td>
</tr>
<tr>
<td>IgG I (\geq 1:1024)</td>
<td>6</td>
<td>587</td>
<td>62% (44–77)</td>
</tr>
<tr>
<td>IgG I (\geq 1:4096)</td>
<td>6</td>
<td>587</td>
<td>24% (11–42)</td>
</tr>
<tr>
<td>IgG I (\geq 1:1024)</td>
<td>3 and 6</td>
<td>561</td>
<td>38% (22–56)</td>
</tr>
<tr>
<td>IgG II (\geq 1:4096)</td>
<td>3</td>
<td>622</td>
<td>84% (66–94)</td>
</tr>
<tr>
<td>IgG II (\geq 1:4096)</td>
<td>6</td>
<td>587</td>
<td>94% (79–99)</td>
</tr>
<tr>
<td>IgG II (\geq 1:4096)</td>
<td>3 and 6</td>
<td>561</td>
<td>84% (66–94)</td>
</tr>
<tr>
<td>IgG I (\geq 1:1024) and IgG II (\geq 1:4096)</td>
<td>6</td>
<td>587</td>
<td>59% (41–75)</td>
</tr>
</tbody>
</table>
incubation period for chronic Q fever. Additional cases of chronic Q fever may yet become apparent in the group that we studied. For this reason, the present study is being continued and includes patients with acute Q fever from the unprecedented outbreak of 2009.

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