High *Coxiella burnetii* DNA Load in Serum during Acute Q Fever Is Associated with Progression to a Serologic Profile Indicative of Chronic Q Fever

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PCR is very effective in diagnosing acute Q fever in the early stages of infection, when bacterial DNA is present in the bloodstream but antibodies have not yet developed. The objective of this study was to further analyze the diagnostic value of semi-quantitative real-time PCR (qPCR) in diagnosing acute Q fever in an outbreak situation. At the Jeroen Bosch Hospital, in 2009, qPCR testing for *Coxiella burnetii* DNA was performed for 2,715 patients suspected of having acute Q fever (positive, $n = 385$; negative, $n = 2,330$). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the qPCR assay were calculated for patients with negative qPCR results with a follow-up sample obtained within 14 days ($n = 305$) and qPCR-positive patients with at least one follow-up sample ($n = 369$). The correctness of the qPCR result was based on immunofluorescence assay results for samples submitted for qPCR and follow-up testing. The sensitivity of the Q fever qPCR assay was 92.2%, specificity 98.9%, PPV 99.2%, and NPV 89.8%. Patients who later developed serologic profiles indicative of chronic Q fever infection had significantly higher *C. burnetii* DNA loads during the acute phase than did patients who did not ($P < 0.001$). qPCR testing is a valuable tool for the diagnosis of acute Q fever and should be used in outbreak situations when the onset of symptoms is <15 days earlier. Special attention is needed in the follow-up monitoring of patients with high *C. burnetii* DNA loads during the acute phase, as this might be an indicator for the development of a serologic profile indicative of chronic infection.

Infection by the intracellular bacterium *Coxiella burnetii*, which is transmitted to humans from infected animals (in particular, small ruminants), causes Q fever (1). During four consecutive years starting in 2007, the largest Q fever epidemic recorded to date occurred in the south of the Netherlands (>4,000 notified cases) (2, 3). The affected area has a high density of dairy goats, which were considered the primary source of infection (3–6).

The gold standard for serologic diagnosis of an infectious disease is either seroconversion or a 4-fold increase in antibody titers. The reference test for serologic diagnosis of Q fever is the immunofluorescence assay (IFA) (1, 7). Antibodies against phase II antigens are expressed during acute infections, whereas high levels of antibodies against phase I antigens are detected in patients with chronic Q fever (8–10). IgM antibodies against phase II antigens (IgM phase II) are the first antibodies to appear after infection, followed by IgG antibodies against phase II antigens (IgG phase II) and IgM antibodies against phase I antigens (IgM phase I) and finally IgG antibodies against phase I antigens (IgG phase I) (8). However, there is a lag in antibody responses of 7 to 15 days after the onset of symptoms, which is an important disadvantage to note for serology, as it is the primary method of diagnosis for acute Q fever (1). Another difficulty in diagnosing acute Q fever is the persistence of IgM phase II antibodies (11).

C. burnetii DNA-specific PCR can be an effective tool to diagnose acute Q fever before seroconversion takes place, as *C. burnetii* DNA has been found in blood samples from patients in that stage of the disease (12). It is unclear whether cell-free DNA or intact bacteria are generally present in blood. The latter has been demonstrated by a well-documented case of Q fever transmitted by blood transfusion (13). The sensitivities of various *C. burnetii* PCR assays to detect Q fever within 2 weeks after disease onset range from 26% to 98% (12, 14, 15).

Acute Q fever infections can remain asymptomatic or present as a flu-like illness, pneumonia, or hepatitis (1, 10, 16). Chronic Q fever develops in approximately 2% of symptomatic acute Q fever cases (17). Patients with preexisting valvular disease, aneurysms, or vascular grafts, immunocompromised patients, and pregnant women are most at risk for chronic infection (1). The diagnosis of chronic Q fever is difficult and relies mainly on serologic findings, blood or tissue PCR assay results, clinical findings, the presence of risk factors, and the results of diagnostic imaging techniques (18). Nevertheless, as chronic Q fever has high morbidity and mortality rates if left untreated, early detection and treatment are important (19).

Delays in treatment and failure to diagnose acute Q fever may lead to prolonged morbidity and increased hospital admission rates (3, 20–22). In 2007 in the Netherlands, the percentage of hospitalized patients was 46% and there was a considerable period of time between the actual onset of the epidemic and the public

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health department knowing of its presence ([3]). Routine laboratory diagnostic tests based on seroconversion in convalescent-phase serum samples and the unawareness of medical staff members resulted in relatively late diagnoses; both of those factors improved in subsequent years, which reduced the diagnostic delays ([3], [23], [24]).

The majority of diagnostic samples from the Dutch epidemic were submitted to our laboratory, which is located in the center of the epidemic area (Department of Medical Microbiology and Infection Control, Jeroen Bosch Hospital [JBH], 's-Hertogenbosch, the Netherlands). The laboratory serves a catchment area of approximately 500,000 persons in a semirural district, supporting two hospitals and surrounding general practitioners (GPs). In 2009, the JBH received over 18,000 requests for Q fever diagnostic tests, with a maximum of 182 requests on 1 day, and diagnosed more than 1,300 Q fever cases. In order to improve laboratory logistics during the outbreak situation, an algorithm was developed to cope with the large number of submitted samples. IgM phase II tests, enzyme-linked immunosorbent assays (ELISAs), and PCR assays were included as routine procedures within this algorithm (see Materials and Methods for more details) ([25]).

The objective of this study was to further analyze the diagnostic value of semiquantitative real-time PCR (qPCR) in the diagnosis of acute Q fever in an outbreak situation. Additionally, cycle threshold (C_T) values (i.e., bacterial DNA load) in the acute phase were studied.

MATERIALS AND METHODS

In this study, all samples submitted to our laboratory in 2009 by GPs and hospital physicians requesting acute Q fever diagnostic tests for which qPCR testing was performed were investigated. The *C. burnetii* qPCR test, together with its analytical sensitivity and specificity, has been described previously ([12], [26]).

Samples were excluded if an external laboratory requested the test, if qPCR was performed to check for chronic Q fever infection, or if acute Q fever had been diagnosed previously. The main reason for not including samples from external laboratories was that, in most cases, only one sample per patient was received for additional qPCR testing to identify chronic Q fever.

GPs and hospital physicians submitting samples either supplied or did not supply the requested date of disease onset. Diagnostic evaluations for patients suspected of having acute Q fever were performed according to the diagnostic algorithm that was established in our laboratory in 2009 ([25]). In brief, all serum samples were initially screened with a qualitative ELISA for IgM phase II (MII screen), according to the manufacturer's instructions (Institut Virion/Serion GmbH, Würzburg, Germany), with a DSX automated ELISA processing system (Dynex Technologies, Chantilly, VA). qPCR was performed on all MII screen-negative samples from GP-referred patients with a period of ≤14 days between the onset of illness and the acquisition of the serum sample, as well as all MII screen-negative samples from hospital-admitted patients. When the date of disease onset was missing from requests from GPs, qPCR was not performed and a second sample was requested. In cases in which qPCR was performed and the result was negative, a second sample (after 14 days) also was requested ([25]). IFAs for IgM and IgG phase I and phase II (Focus Diagnostics, Inc., Cypress, CA) were performed on all MII screen-positive and borderline-positive serum samples, according to the manufacturer's instructions. Titers of 1:32 or greater were considered positive ([25]). After a patient was diagnosed with acute Q fever, follow-up samples were requested at 3, 6, and 12 months, to monitor the development of chronic Q fever ([27]).

C. burnetii qPCR targeting the multicopy IS*1111a* gene ([26]) was performed in duplicate as described (the input volume of serum was 500 μl

and the elution volume was 60 μl; for retesting, the input volume of serum was 500 μl and the elution volume was 25 μl) ([12]). qPCR results were considered positive when at least one C_T value was <45. To avoid contamination of qPCR assays with genomic *C. burnetii* DNA or amplicons from previous reactions, the preparation of the PCR reagents, the isolation of DNA, and the amplification by qPCR (closed system) were carried out in three separate rooms designated for those activities. A mock isolation (negative control [NC], i.e., simulated isolation that mimics the process of sample handling) and a water control (no-template control [NTC]) were included in each run. Among the 347 NC plus 315 NTC samples that were analyzed in 2009, we once detected a *C. burnetii* signal with a C_T of 36.22 in an NC sample that was in the qPCR plate next to a relatively high-level positive sample (C_T of 28.58); all other 661 control samples tested negative.

To assess whether qPCR-positive patients actually had acute Q fever, IFA results for one or more follow-up samples were evaluated. To classify a positive qPCR result as correct, IgG phase I and/or phase II seroconversion with a titer of ≥1:64 or a ≥4-fold increase in the IgG phase II titer needed to be detected in a follow-up sample. Due to the enormous number of samples that were submitted to our laboratory for Q fever diagnostic tests (over 18,000 samples in 2009), IFA results sometimes were not titrated but were recorded only as "positive." In addition, IgG phase I and II levels were not always determined in samples submitted for qPCR testing with a suspicion of acute Q fever (when ELISA/IFA IgM phase II results were negative [25]). Therefore, to establish seroconversion rates, we assumed that the IgM phase II-negative qPCR samples were also negative for IgG phase I and II. To test this assumption, we analyzed the IgG phase I and II status of 16 qPCR-positive IgM phase II-negative patient samples. All 16 samples tested negative for IgG phase I and II. Patients with inconclusive serologic results, patients with nonspecific antibody responses, and patients with detectable IgG antibodies for whom IgG seroconversion or a 4-fold IgG phase II titer increase could not be confirmed were excluded from this analysis. To evaluate whether negative qPCR results correctly identified patients without acute Q fever, we included all qPCR-negative samples from patients who submitted a second serum sample within 14 days after the first sample. Thus, we excluded qPCR-negative patients with no follow-up sample, patients with IgG phase II antibodies present in the diagnostic qPCR-negative sample without a 4-fold IgG phase II titer increase, and patients with a follow-up sample submitted ≥15 days after the first sample, as we were unable to assess whether the qPCR results were correct for those patients. To illustrate the latter case, a patient with a follow-up sample submitted ≥15 days after the first sample could in theory be infected after the first sample was obtained, as qPCR results are positive for only approximately 14 days after the onset of symptoms.

Additionally, C_T values obtained during the acute phase of infection for qPCR-positive patients were investigated in relation to age, time after onset of symptoms, IgG phase I titer at 12 months after diagnosis, and development of a serologic profile for chronic Q fever. A serologic profile indicative of chronic Q fever was defined as an IgG phase I titer of ≥1:1,024 in serum obtained approximately 12 months after the qPCR-positive sample. Proven chronic Q fever was defined as an IgG phase I titer of ≥1:1,024 and a positive qPCR result observed within approximately 12 months after the acute Q fever diagnosis. To estimate the *C. burnetii* DNA load (genome equivalents [GEq] per milliliter) in serum from duplicate C_T values, the following formula was used: *C. burnetii* DNA load (GEq/ml) = $10^{(C_T - 35.433)/-3.3951} \times 2 \times 6 \times (100/57)$. The first part of the formula is based on the dilution experiment described previously ([12]), the factor 2 on 500 μl serum input for DNA isolation, the factor 6 on qPCR input of 10 μl of a 60-μl elution volume, and the factor 100/57 on correction for the easyMAG isolation efficiency for bacterial DNA of 57% ([28]).

Descriptive characteristics were investigated by calculating relative frequencies, medians, and interquartile ranges (IQRs). Chi-square tests were performed to assess significant differences in gender distribution, and Mann-Whitney *U* tests were used for age distribution and C_T values.

TABLE 1 Characteristics of patients with *Coxiella burnetii* real-time PCR results obtained in 2009 to assess the presence of acute Q fever infection

qPCR result	No. (%) male	Median age (IQR) (yr)
Total (*n* = 2,715)	1,412 (52)	49 (34–64)
Positive (*n* = 385)	264 (69)[a]	50 (38.5–60)[b]
Negative (*n* = 2,330)	1,151 (49)[a]	49 (33–64)[b]

[a] Positive qPCR result versus negative qPCR result: $P < 0.001$, chi-square test.
[b] Positive qPCR result versus negative qPCR result: $P = 0.563$, Mann-Whitney U test. IQR, interquartile range.

TABLE 2 Numbers of patients included in diagnostic value analysis for the *Coxiella burnetii* real-time PCR assay for the diagnosis of acute Q fever

	No. of patients		
qPCR result	Infection in follow-up sample[a]	No infection in follow-up sample[b]	Total
Positive	366	3	369
Negative	31	274	305
Total	397	277	674

[a] IgG phase I and/or phase II seroconversion with a titer of ≥1:64 in a follow-up sample or a ≥4-fold increase in the IgG phase II titer in a follow-up sample.
[b] No IgG phase II antibodies (<1:64) detected in one or more follow-up samples.

To investigate the diagnostic value of qPCR in diagnosing acute Q fever, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and corresponding 95% confidence intervals (Wilson score intervals). Furthermore, the cumulative percentage of patients developing a chronic Q fever serologic profile for different *C. burnetii* DNA loads in the acute phase was calculated. Spearman's rank correlation (r_s) coefficients were calculated to determine potential correlations between age, time after symptom onset, and IgG phase I titer at 12 months after diagnosis. Data were analyzed using SPSS Statistics version 19.0.0 (SPSS Inc.) and Microsoft Excel (Microsoft Corp.).

RESULTS

In 2009, qPCR was performed at the JBH for 3,040 patients. After the exclusion of 325 patients because of requests by external laboratories (*n* = 267), checking for chronic infection (*n* = 36), and previously diagnosed acute Q fever infections (*n* = 22), 2,715 patients remained. Table 1 presents characteristics of these patients. The date of symptom onset provided by GPs and hospital physicians submitting samples was available for 68% (*n* = 1,836). Negative qPCR responses were observed for 2,330 patients, and 385 patients had positive qPCR results. A statistically significant difference was observed in gender distribution between qPCR-positive and -negative patients ($P < 0.001$) (Table 1).

Of the 2,330 patients with negative qPCR results, 1,299 (56%) did not have a follow-up serum sample taken and 714 (31%) had their follow-up sample taken ≥15 days after the first sample. Moreover, 12 patients were excluded from the diagnostic value analysis because they had inconclusive serologic results (*n* = 3), nonspecific serologic responses (*n* = 3), already evident antibodies in a previous sample (i.e., qPCR was unnecessary [*n* = 2]), IgG phase II present in the qPCR-negative sample without a 4-fold IgG phase II titer increase (*n* = 2), or previously resolved Q fever infection (*n* = 2). In the end, 305 qPCR-negative patients remained available for analysis (male, *n* = 154 [51%]; median age, 52 years [IQR, 39 to 62 years]). Nine (2%) of the 385 qPCR-positive patients did not provide a follow-up sample, six patients had detectable IgG phase II antibodies although IgG seroconversion or a 4-fold IgG phase II titer increase could not be confirmed with the data available, and one qPCR-positive patient was excluded because of inconclusive serologic results. Subsequently, 369 qPCR-positive patients remained available for the analysis (male, *n* = 250 [68%]; median age, 50 years [IQR, 38.5 to 60 years]), including 366 based on seroconversion and 3 based on a 4-fold increase in antibody titer.

Table 2 presents the outcome of the diagnostic value analysis for the qPCR assay for the diagnosis of acute Q fever. Seventy-two (20%) of the 369 qPCR-positive patients had detectable IgM phase II measured by IFA and/or ELISA in their qPCR-positive samples. Table 3 presents the diagnostic values found with the numbers presented in Table 2. The sensitivity was 92.2%, specificity 98.9%, PPV 99.2%, and NPV 89.8%. The three false-positive samples (C_T values [duplicate] of 36.90 and 37.39, 36.38 and undetectable, and 34.32 and 35.65) were retested; the first two showed inhibition of the qPCR and the third was again positive (C_T values of 33.61 and 31.60). The follow-up samples for the patient who repeatedly tested positive showed no IgG phase I and low IgG phase II levels (1:32) up to 1 year after the qPCR-positive sample and thus did not meet our definition of confirmed acute Q fever infection.

Of the 31 patients with false-negative qPCR test results (all based on seroconversion), 19 patients had negative MII screen results, 10 patients had positive or ambiguous MII screen results and positive IgM phase II IFA results, and 1 patient had a positive IgM phase II IFA result; for 1 patient, only qPCR was performed on the first sample but the second sample, submitted 5 days later, was IFA IgM phase II, IgM phase I, and IgG phase II positive. Four of the 12 patients for whom IgM phase II antibodies (ELISA and/or IFA) were detected also had detectable IgM phase I antibodies, while IgG phase II results were negative for all 12 patients. Four of the 31 patients never developed IgG phase I antibodies during the follow-up period.

Table 4 and Fig. 1 show the relationships observed between C_T values and age, time after illness onset, and IgG phase I titers approximately 12 months after diagnosis. Significant correlations were found for the number of days after the onset of illness and IgG phase I titers at 12 months after diagnosis (Table 4). Significant weak negative correlations also were observed for IgG phase I and II antibody levels at 3 and 6 months after diagnosis and for IgG phase II antibody levels at 12 months after diagnosis (data not shown). Additionally, it was observed that 107/369 (30%) of qPCR-positive patients had low circulating *C. burnetii* DNA loads (<10 GEq/ml) at the time of diagnostic sampling.

TABLE 3 Diagnostic value of the *Coxiella burnetii* real-time PCR assay for the diagnosis of acute Q fever

Diagnostic value	% (95% confidence interval)[a]
Sensitivity	92.2 (89.1–94.4)
Specificity	98.9 (96.9–99.6)
Positive predictive value	99.2 (97.6–99.7)
Negative predictive value	89.8 (85.9–92.8)
Confirmed acute Q fever infection rate	58.9 (55.2–62.6)

[a] Wilson score interval.

TABLE 4 Spearman rank correlation coefficients for mean C_T values for duplicate *Coxiella burnetii* real-time PCR tests in relation to age, number of days after illness onset, and IgG phase I titers 12 months after diagnosis

Parameter analyzed	No. of patients	Spearman rank correlation coefficient $(r_s)^a$	P^b
Age	366	−0.005	0.924
Days after illness onset	249	0.232	<0.001
IgG phase I titer 12 mo after diagnosis	312	−0.338	<0.001

^{a} Spearman rank correlation coefficient for the relationship between C_T values and the parameter indicated.
^{b} Two-tailed test.

Follow-up serum samples obtained approximately 12 months after diagnosis were available for 85% (312/369) of the patients with true-positive qPCR results. Nineteen of those patients developed serologic profiles indicative of chronic Q fever. One additional patient developed proven chronic Q fever without a 12-month follow-up sample (deceased). Overall, 20/313 (6.4%) had serologic profiles indicative of chronic Q fever, and one additional patient with a serologic profile indicative of chronic Q fever was observed among the patients with false-negative qPCR results, with IgM phase I and II antibodies present in the diagnostic qPCR-negative sample (Table 5). The median C_T value for the 20 patients diagnosed with positive qPCR results was 28.90 (IQR, 25.60 to 35.55), which was significantly different from the median C_T value for the 293 patients without serologic profiles for chronic Q fever (35.01 [IQR, 33.00 to 39.56]; $P < 0.001$). Furthermore, 10 of those 20 patients were among the patients with the 17 lowest C_T values found ($C_T \leq 28.20$) among all 366 patients (median C_T value for those 10 patients, 25.65 [IQR, 23.10 to 27.31]). Figure 2 presents the cumulative percentages of patients who developed serologic profiles indicative of chronic Q fever with different *C. burnetii* DNA loads in the acute-phase serum samples. Eighty-six percent of patients with *C. burnetii* DNA loads above 10,000 GEq/ml in the acute phase developed serologic profiles indicative of chronic Q fever.

DISCUSSION

This study presents data for a large number of patients who were tested with qPCR for *C. burnetii* DNA during an acute Q fever outbreak. Previously, we showed that the qPCR assay used in our department detects *C. burnetii* DNA in virtually all sera of seronegative patients with acute Q fever but results rapidly become negative when the antibody response develops (12). However, the positive and negative predictive values of the *C. burnetii* qPCR have not been examined thus far.

For the Q fever diagnostic work-up, we followed the algorithm described previously (25). IgG phase I and II levels were not determined in the samples submitted for qPCR testing when ELISA/IFA IgM phase II results were negative. Therefore, to establish seroconversion rates, we assumed that the IgM phase II-negative qPCR samples were also negative for IgG phase I and II. This assumption was reasonable since we were dealing with a high-incidence outbreak in a low-prevalence area; therefore, the chances for chronic Q fever in this patient cohort were very small. IFA retesting of the qPCR-positive samples from patients who had IgG phase I titers of ≥1:1,024 in the first follow-up sample ($n =$
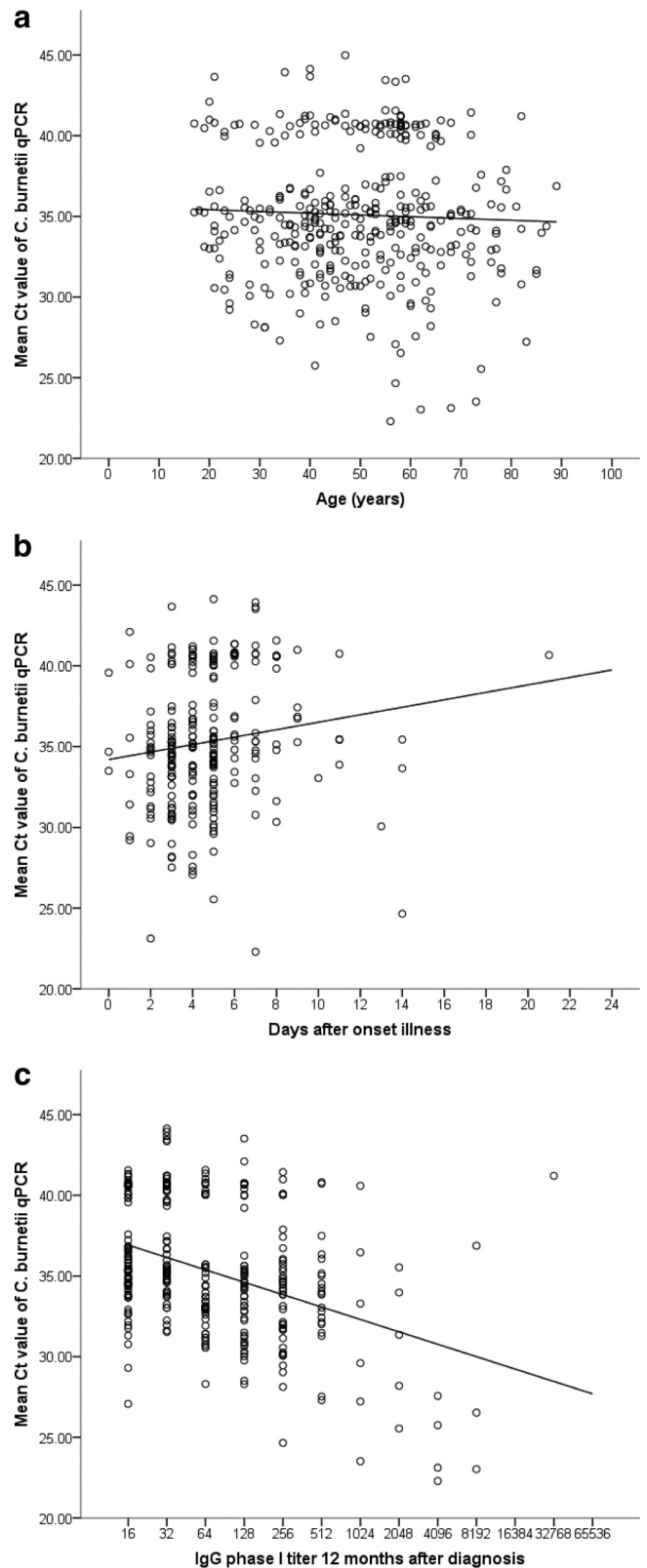
FIG 1 Relationships between the mean cycle threshold (C_T) values of duplicate *Coxiella burnetii* real-time PCR tests and age ($n = 366$) (a), days after the onset of illness ($n = 249$) (b), and IgG phase I titers at 12 months after diagnosis ($n = 312$) (c).

TABLE 5 Clinical and serologic findings for the 21 patients with serologic profiles indicative of chronic Q fever 12 months after acute Q fever infection

Patient no.	Mean C_T value at acute Q fever diagnosis	Serologic results 12 mo after diagnosis[a]			Clinical findings	Chronic Q fever category[b]
		IgG phase I titer	IgG phase II titer	qPCR result		
1	22.30	4,096	8,192	Negative	No risk factors[c]	C
2	23.03	>4,096	>4,096	Negative	No risk factors[c]	C
3	23.12	4,096	4,096	Positive	Focus unknown	A
4	23.52	1,024	1,024	Negative	Granulomatous abnormality in lung	B
5	25.55	2,048	2,048	Negative	No risk factors[c]	C
6	25.75	4,096	4,096	Negative	No risk factors[c]	C
7	26.53	>4,096	>4,096	Negative	Infected vascular prosthesis	A
8	27.23	1,024	>4,096	Negative[d]	Endocarditis	A
9	27.57	4,096	16,384	Negative	Endocarditis	B
10	28.20	2,048	>4,096	Negative	No risk factors[c]	C
11	29.60	1,024	>4,096	Negative	No risk factors[c]	C
12	31.36	2,048	4,096	Negative	No risk factors[c]	C
13	33.29	1,024	1,024	Negative	No risk factors[c]	C
14	33.98	2,048	>4,096	Negative	Deceased with several comorbidities	Unknown
15	35.53	2,048	4,096	Negative	Endocarditis	B
16	35.56	NA	NA	NA	Kidney transplant; deceased within 12 mo after diagnosis, had positive qPCR result for follow-up serum sample	A
17	36.46	1,024	2,048	Negative	No risk factors[c]	C
18	36.88	>4,096	>4,096	Positive	Infected aneurysm	A
19	40.59	1,024	>4,096	Negative	No risk factors[c]	C
20	41.21	32,768	65,536	Positive	Endocarditis	A
21	False-negative qPCR result, positive IgM phase I and II results for diagnostic qPCR-negative sample	1,024	1,024	Negative	No risk factors[c]	C

[a] NA, not applicable.
[b] A, proven chronic Q fever; B, probable chronic Q fever; C, possible chronic Q fever, according to Wegdam-Blans et al. (18).
[c] No cardiac or vascular risk factors.
[d] A follow-up serum sample obtained >12 months after the acute Q fever diagnosis was qPCR positive.

18; 14 samples were available, and 4 samples were unavailable) indeed showed that none had detectable IgG phase I or II antibodies in the initial qPCR-positive sample.

In this study, we have been quite conservative in assigning patients to the true-positive and true-negative categories, and the presented diagnostic values might be underestimations. For example, patients with negative qPCR and positive MII screen and/or IFA IgM phase I and II results were assigned to the false-negative qPCR category, although the qPCR finding probably was a true-negative result as IgM antibodies were already present. If we had investigated the diagnostic value of the qPCR assay before the presence of antibodies, then the 12 patients with detectable IgM phase II antibodies (IFA and/or ELISA) in their qPCR-negative samples would not have been classified in the false-negative category and the reported sensitivity would be an underestimation.

We were not able to include all qPCR-negative patients as the majority of qPCR-negative patients did not submit a follow-up sample or it was submitted too late. Usually, general practitioners and patients are less likely to send in requested follow-up samples when symptoms have resolved or another cause of illness has been found. They are more likely to send in requested follow-up sam-

ples when undiagnosed symptoms persist. Nevertheless, some bias might have occurred in this study as we missed some cases with false-negative qPCR results. Thus, the sensitivity and, to a lesser extent, the negative predictive value presented here are overestimations.

We found three patients with false-positive qPCR results, one of whom also had positive retest results. The other two samples unfortunately showed inhibition of the qPCR in the retest, probably due to the smaller elution volume (25 µl in the retest versus 60 µl in the first test) and therefore higher concentrations of inhibiting substances after DNA isolation. Although no clear seroconversion was observed for the patient with the consistent positive qPCR test results, a low positive IgG phase II titer (1:32) was found in two follow-up samples.

Positive qPCR results for blood donors who do not develop a clear antibody response were observed previously by Hogema et al. (29). Those authors found that three of the six blood donors with qPCR-positive results did not have seroconversion measured by IFA (29). Since qPCR is an extremely sensitive technique for the detection of DNA, contamination of patient samples during processing always poses a risk for false-positive test results. This might have been the case for our three false-positive patients.

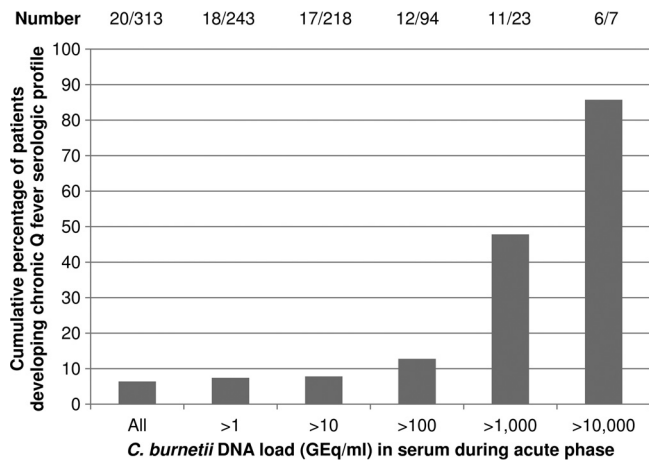Number 20/313   18/243   17/218   12/94   11/23   6/7

FIG 2 Cumulative percentages of patients who developed serologic profiles indicative of chronic Q fever infection, classified according to the estimated *C. burnetii* DNA load in the acute-phase serum samples. Patients were included only when a serum sample obtained approximately 12 months after the qPCR-positive sample was available ($n = 312$) or when a proven chronic Q fever infection was observed <12 months after the acute Q fever diagnosis ($n = 1$). Chronic Q fever infection was determined on the basis of an IgG phase I titer of ≥1:1,024 in a serum sample obtained approximately 12 months after the qPCR-positive sample or observation of a proven chronic Q fever infection (IgG phase I titer of ≥1:1,024 and a positive qPCR result) <12 months after the acute Q fever diagnosis.

It was observed that often low circulating *C. burnetii* DNA loads were present in qPCR-positive samples (C_T values of >36.50 correspond to loads of <10 GEq/ml). This indicates that high test sensitivity is important for detection of such low *C. burnetii* loads. These low DNA loads may well contribute to the differences in the reported sensitivities of various PCR assays (26% to 98% [12, 14, 15]).

We excluded patients for whom qPCR was performed in relation to chronic Q fever infection, as we assessed performance of the qPCR assay in the acute phase. qPCR results can be positive for Q fever patients in the acute phase, which occurs in the first 2 weeks after disease onset (12), and for patients with chronic Q fever (18). After the acute phase, qPCR results become negative at the time of antibody development. qPCR results can again become positive for chronic Q fever patients when IgG phase I antibodies are present (18). It is not known whether all patients who develop chronic Q fever have a period between the acute phase and the chronic phase during which qPCR results are negative. Some patients may not be able to control the acute infection at all, directly progressing to a chronic stage with persistence of qPCR positivity.

Although weak, a significant positive correlation was observed between C_T values and days after the onset of illness, as well as a significant negative correlation between C_T values and IgG phase I titers at 12 months after diagnosis. A statistically significantly lower C_T value (i.e., a higher bacterial DNA load) during acute infection was found for patients who later developed serologic profiles indicative of chronic Q fever infection than for patients who did not. Only four of the 10 patients with C_T values of ≤28.20 (corresponding to approximately 2,900 GEq/ml) had clear risk factors for chronic Q fever (Table 5). The 21 patients with serologic profiles indicative of chronic Q fever (including the patient with a false-negative qPCR result) were classified as described previously (18), which resulted in six cases of proven chronic Q fever,

three probable cases, and 11 possible cases, while one case could not be classified (the patient was deceased with several comorbidities, with negative qPCR results for the 12-month follow-up sample). Of patients with *C. burnetii* DNA loads of >1,000 GEq/ml, 48% developed serologic profiles indicative of chronic infection, which increased to 86% for patients with loads of >10,000 GEq/ml. Thus, a low C_T value/high bacterial DNA load, which unfortunately is measurable only in the first 2 weeks after disease onset, might be an additional risk factor for developing a serologic profile indicative of chronic infection, independent of already known risk factors. Additional patients with low C_T values might be identified in new outbreak situations or by retrospective testing of stored serum samples obtained from acute Q fever patients <15 days after symptom onset.

Molecular tests are generally sensitive to carryover and cross-contamination, which may lead to false-positive test results. Although the three false-positive test results in our study were probably due to contamination, there are several indications that cross-contamination was not a common phenomenon in the *C. burnetii* qPCR testing in our laboratory. First, as mentioned in Materials and Methods, we used separate rooms for the preparation of the PCR reagents, the isolation of DNA, and the amplification by qPCR (closed system). Second, the simulated isolations that mimicked the process of sample handling and the water controls included in each run systematically tested negative. Third, our laboratory participated in an interlaboratory evaluation/quality check of the qPCR assay for *C. burnetii* (26). No contamination was seen for the qPCR assay with serum samples. Further, if cross-contamination had occurred, then the samples with the lowest *C. burnetii* burdens would be most suspected of being contaminated. Of the 369 patients with positive qPCR results in this study, 281 patients had duplicate C_T values of <40, 78 patients had one C_T value of <40 and one negative qPCR result, two patients had one C_T value of <40 and one between 40 and 45, and eight patients had one C_T value between 40 and 45 and one negative C_T value. All except 1 of the 88 patients with the lowest *C. burnetii* loads developed antibodies against *C. burnetii* during the follow-up period. The single patient who did not develop a serologic response was considered to have a false-positive result. If our high sensitivity reflected contamination, then we would have had more false-positive findings.

In conclusion, qPCR is an effective and valuable tool for the diagnosis of acute Q fever in outbreak situations. *C. burnetii* qPCR testing with serum samples should be included routinely in the diagnostic work-up for patients with suspected acute Q fever in outbreak situations when the onset of symptoms is <15 days earlier. Special attention is needed in the follow-up monitoring of patients with high *C. burnetii* DNA loads (i.e., low C_T values) during the acute phase of Q fever infection, as this might be an indicator for the development of a serologic profile indicative of chronic Q fever.

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