

Molecular Detection of *Coxiella burnetii* in the Sera of Patients with Q Fever Endocarditis or Vascular Infection

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In the absence of a specific diagnosis based on serology, chronic Q fever is inevitably fatal. However, diagnosis is often delayed because the test is not widely available. To shorten the diagnostic delay, we adapted a nested-PCR assay with serum as a template and the LightCycler as a thermal cycler, termed LCN-PCR. We retrospectively and prospectively applied this method to samples from 48 patients diagnosed with Q fever endocarditis or vascular infection and to samples from 100 controls with endocarditis caused by other microorganisms. We also prospectively applied this technique to samples from 30 patients treated for a Q fever endocarditis and to samples from 13 patients with a convalescent acute Q fever with ambiguous immunoglobulin G (IgG) phase I titer. LCN-PCR had a specificity of 100%. It was positive only in samples from patients with evolutive Q fever, as none of the samples from patients with a treated chronic Q fever or with a convalescent acute Q fever presented positive results. When performed prospectively on recently stored sera, the sensitivity of LCN-PCR is 64% (7 of 11 samples; $P = 0.004$), but the efficiency of LCN-PCR was dramatically altered by the storage of specimens at -20°C . High IgG phase I titers decreased the sensitivity of LCN-PCR. A significant difference was observed among LCN-PCR results for sera with IgG phase I titers of $\geq 1:25,600$ compared to sera with IgG phase I titers of $< 1:25,600$ (0 of 15 samples versus 13 of 33 samples; $P = 0.004$). In patient samples with titers below 1:25,600 tested prospectively, sensitivity was 100% (7 of 7). The LCN-PCR assay may be helpful in establishing an early diagnosis of chronic Q fever.

Q fever is an ubiquitous zoonosis caused by *Coxiella burnetii* (18). Q fever can cause acute or chronic infection (17, 18, 22). Acute Q fever is asymptomatic in 60% of infected persons. In symptomatic patients, the clinical presentation is polymorphic and nonspecific. The major forms of acute Q fever described to date are febrile illness, atypical pneumonia, and hepatitis. Acute Q fever is usually mild (20, 22), but patients with cardiovascular abnormalities are at risk of developing chronic infection (10, 26). In patients with chronic Q fever, endocarditis is the most common feature, but vascular infections are also observed (7, 13, 23). Q fever has been estimated to represent 3 to 5% of all cases of endocarditis (6).

C. burnetii is a short and pleomorphic strictly intracellular bacillus which presents a variation of phase comparable to the smooth-rough variation described for the *Enterobacteriaceae*. In nature, *C. burnetii* expresses only the phase I antigen (equivalent to the smooth phase) (5, 27). This phase is observed in infected humans, animals, and arthropods and represents the infectious form of the bacterium. The phase II variant is obtained after several passages on embryonated eggs or cell cultures and is less virulent. The reversion to phase I is made possible by inoculation into the animal host. In phase I, the lipopolysaccharide is present in its entire length, whereas in phase II, we observed lipopolysaccharide which contains fewer sugars in the lateral chain. The reservoir of *C. burnetii* includes mammals, birds, and arthropods, mainly ticks (18). *C. burnetii* is shed in urine, feces, milk, and especially the birth products of

mammals. The usual source of human infection is farm animals. This organism is highly infectious and is currently considered a potential warfare agent, classified as a category B biological agent by the Center for Diseases Control and Prevention (4, 8, 16, 19). In humans, infection most often results from inhalation of contaminated aerosols from amniotic fluid, placenta, or contaminated wool (16, 18). The diagnosis of Q fever relies mainly on serological examination, the most commonly used method being the indirect immunofluorescence assay (29). Acute and chronic infections are characterized by different serological profiles (18). For chronic Q fever, the best tool is the analysis of antibodies directed against the phase I antigen of *C. burnetii*, which is not always available on the usual commercialized tests. As cutoff values in the immunofluorescence assay, a phase I immunoglobulin G (IgG) titer superior or equal to 1:800 is recommended for the diagnosis of chronic Q fever (14). This value allows excellent sensitivity but a loss of specificity. A titer of 1:1,600 exhibits a better specificity but a lower sensitivity. Therefore, a confirmation test for sera positive at a titer of 1:800 may be useful. The development of a bacterial DNA detection approach in serum is therefore of interest. However, typical one-step PCR amplification from such a specimen may not achieve sufficient sensitivity. Recently, to increase our detection threshold, two efficient and rapid nested-PCR assays using serum as a template and the Light-Cycler (Roche Diagnostics, Basel, Switzerland) as a thermal cycler, named LCN-PCR, have been developed in our laboratory for the diagnosis of acute Q fever and for the diagnosis of *Bartonella* endocarditis (19, 31). Here, our aim was to estimate whether LCN-PCR would be suitable for the diagnosis of Q fever endocarditis and vascular infections. With this goal, the sensitivity of this technique was evaluated with sam-

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TABLE 1. List of the sera tested by the LCN-PCR assay

| Patient group (no. of patients) and type of analysis | Disease, infection, or treatment (no. of patients) | No. of sera analyzed | |
|--|--|--|----|
| Patients with evolutive chronic Q fever (48) | Retrospective ^a | Q fever endocarditis (35) | 35 |
| | | Q fever vascular infection (2) | 2 |
| | Prospective ^b | Q fever endocarditis (9) | 9 |
| | | Q fever vascular infection (2) | 2 |
| Patients without evolutive chronic Q fever (43) | Prospective | Treated for Q fever endocarditis for 1 mo or more (30) | 30 |
| | | Convalescent acute Q fever presenting ambiguous titers with IgG phase I titer that was $\geq 1:800$ (13) | 13 |
| | | | |
| Control (100) | | | |
| Restrospective | Endocarditis caused by a microorganism other than <i>C. burnetii</i> (100) | 100 | |

^a Sera were stored at -20°C .

^b Sera were tested less than 1 month after sampling.

ples from patients with endocarditis and vascular infections due to *C. burnetii*, and its specificity was evaluated with samples from patients with endocarditis due to other microorganisms and in patients without chronic Q fever and with an IgG phase I titer equal to or greater than 1:800. Finally, the suitability of this technique for therapeutic follow-up was evaluated on samples from patients treated for chronic Q fever.

MATERIALS AND METHODS

Patients. As a reference center for the diagnosis of rickettsial diseases, our laboratory receives serum samples from throughout France and abroad for Q fever diagnosis. For each patient, a standardized questionnaire, including epidemiological and clinical features, is completed by a physician in charge and logged into a database. Patients with Q fever endocarditis are asked questions that are scored according to the diagnostic scale of the Duke Endocarditis Service (Duke University, Durham, N.C.) (9). All the sera tested by the LCN-PCR assay are summarized in Table 1.

Forty-eight sera from 48 patients with evolutive chronic Q fever were included in our study. We selected, from our collection, sera from 37 patients diagnosed as having chronic Q fever for whom at least 200 μl of serum was available. All these sera were stored for >1 month. The other 11 patients included in our study were tested prospectively with fresh serum samples. For stored sera, when several serum specimens for a patient were available, we always used the earliest serum sample at the time of diagnosis, when the antibiotic treatment had not yet begun. For patients for whom the first serum sample was positive by LCN-PCR, we also tested the second serum sampled after 1 month of therapy.

Forty-three sera from 43 patients without evolutive chronic Q fever were also included in our study. To evaluate if this PCR assay could be proposed for the follow-up of patients with chronic Q fever, we prospectively tested 30 sera from 30 patients treated for Q fever endocarditis for 1 month or more with doxycycline and hydroxychloroquine. To verify if our PCR assay specifically detected endocarditis, we also prospectively tested 13 sera from 13 patients with convalescent acute Q fever for whom the IgG phase I titer was equal to or greater than 1:800.

Finally, to estimate the specificity of our PCR assay, we retrospectively tested 100 sera from 100 patients with endocarditis caused by other microorganisms. These control patients were infected as follows: 20 with *Streptococcus viridans*, 18 with *Streptococcus bovis*, 16 with *Staphylococcus aureus*, 10 with *Escherichia coli*, 9 with *Enterococcus faecalis*, 5 with *Bartonella quintana*, 5 with coagulase-negative endocarditis, 4 with *Bartonella henselae*, 3 with *Streptococcus pneumoniae*, 2 with *Neisseria sicca*, 1 with *Streptococcus agalactiae*, 1 with *Gemella morbillorum*, 1 with *Actinobacillus actinomycetemcomitans*, 1 with *Enterobacter cloacae*, 1 with *Klebsiella oxytoca*, 1 with *Stenotrophomonas maltophilia*, 1 with *Haemophilus aphrophilus*, and 1 with *Haemophilus paraphrophilus*.

Diagnostic criteria. The antigenic variation of *C. burnetii* is useful in differentiating acute and chronic disease (14). During acute Q fever, antibodies to phase II antigens predominate. In chronic Q fever, elevated anti-phase I antibodies are detected. In our study, serology by microimmunofluorescence was carried out as previously reported (29). As cutoff values in the immunofluores-

cence assay, a phase I IgG titer of $\geq 1:800$ and/or an IgA titer of $\geq 1:100$ are recommended for the diagnosis of chronic Q fever (14). Endocarditis was defined according to the modified Duke criteria (12). Vascular infections were diagnosed on the basis of the presence of a vascular aneurysm or prosthesis associated with vascular symptoms, fever, a serological profile of chronic Q fever, and no evidence of other infections (9, 13).

Molecular methods. (i) **DNA extraction.** For DNA extraction, two hundred microliters of serum from each sample was used. The DNA was extracted with the QIAamp Blood Kit (QIAGEN, Hilden, Germany) as described by the manufacturer. Fifty microliters of elution buffer was used to resuspend the DNA. Genomic DNAs were stored at 4°C until their use for PCR assays.

(ii) **LCN-PCR.** DNA extraction, mixture preparation, and PCR were performed in different rooms to prevent PCR carryover contamination. No positive control was used, to minimize the risk of lateral contamination with the PCR products amplified in other tubes during the same assay. DNA extracted from serum samples from blood donors was used with every seven specimens as a negative control. Each 20- μl reaction mixture was composed of 4 μl of DNA master SYBR Green; 4.8 μl of 3 mM MgCl_2 ; 1 μl of each of the four primers IS111F1 (5'-TACTGGGTGTTGATATTGC-3'), IS111R1 (5'-CCGTTTCATC CGCGGTG-3'), IS111F2 (5'-GTTAAAGTGATCTACACGA-3'), and IS111R2 (5'-TTAACAGCGCTTGAACGT-3'), each at 0.5 μM ; 5.2 μl of sterile distilled water; and 2 μl of DNA. The four primers target the *hpaAB*-associated repetitive element, which exists in 20 copies in the genome of *C. burnetii* (GenBank accession number AE016828) (25). The IS111F1 and IS111R1 primers, which amplify a 485-bp fragment, were used for the first amplification, and reamplification was performed with the IS111F2 and IS111R2 primers, which target a 260-bp fragment. After an initial denaturation step at 95°C for 8 min, the nested PCR was composed of an initial series of 35 cycles of denaturation at 95°C for 15 s, annealing at 52°C for 5 s, and extension at 72°C for 18 s; this was followed by a second series of 35 cycles of denaturation at 95°C for 15 s, annealing at 48°C for 5 s, and extension at 72°C for 18 s. The amplification was completed by holding for 10 min at 68°C to allow complete extension of the PCR products. As the LightCycler was not able to infer the results from the nested PCR, amplicons from the second amplification were separated by electrophoresis with 1% agarose gels and then purified with a QIAquick PCR purification kit (QIAGEN), as described by the manufacturer.

(iii) **Sequencing of PCR products and sequence analysis.** PCR products were systematically sequenced with the d-Rhodamine Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Coignieres, France) as described by the manufacturer. Sequencing products were resolved with an ABI 3100 automated sequencer (Perkin-Elmer). Sequence analysis was made with the ABI Prism DNA Sequencing Analysis software package, version 3.0 (Perkin-Elmer), and multisequence alignment was performed with CLUSTAL W software, version 1.81 (28). A positive PCR result was based on DNA sequencing.

Serum absorption. The fresh sera with IgG phase I titers equal to or greater than 1:25,600 were also treated with rheumatoid factor (RF) absorbent reagent RF-Absorbent (Dade Behring, Marburg, Germany). The LCN-PCR was then performed on sera before and after absorption. The RF-Absorbent reagent precipitates IgG, forming immune complexes to which the IgM-RF is bound and then removed. Since any pathogen-specific IgG present is also removed by the

TABLE 2. Serological, culture, PCR, and LCN-PCR data for 48 patients with chronic Q fever^a

| Patient no. | MIF assay | | Specific blood culture | PCR of EDTA-treated blood | Cardiovascular biopsy specimen culture | PCR of biopsy specimen | LCN-PCR | Diagnosis |
|-------------|--------------------------------|---------------------------------|------------------------|---------------------------|--|------------------------|---------|--------------------|
| | Ig phase I titer (IgG/IgM/IgA) | Ig phase II titer (IgG/IgM/IgA) | | | | | | |
| 1 | 12,800/100/6,400 | 51,200/1,600/6,400 | NP | - | + | + | - | Endocarditis |
| 2 | 800/25/400 | 1,600/25/400 | NP | NP | NP | NP | - | Endocarditis |
| 3 | 1,600/50/0 | 6,400/100/0 | + | - | NP | NP | + | Endocarditis |
| 4 | 51,200/0/1,600 | 102,400/0/3,200 | NP | NP | NP | NP | + | Endocarditis |
| 5 | 25,600/0/1,600 | 25,600/0/1,600 | NP | NP | NP | NP | - | Endocarditis |
| 6 | 51,200/50/0 | 51,200/50/0 | NP | NP | NP | NP | - | Endocarditis |
| 7 | 25,600/0/3,200 | 51,200/0/6,400 | NP | NP | + | + | - | Vascular infection |
| 8 | 3,200/50/1,600 | 12,800/100/3,200 | - | NP | + | + | - | Endocarditis |
| 9 | 102,400/0/1,600 | 102,400/0/1,600 | - | NP | + | + | - | Endocarditis |
| 10 | 800/0/400 | 800/0/50 | NP | NP | NP | NP | - | Endocarditis |
| 11 | 1,600/0/200 | 3,200/0/400 | NP | NP | - | - | - | Endocarditis |
| 12 | 800/800/200 | 3,200/1,600/200 | NP | NP | NP | NP | - | Endocarditis |
| 13 | 800/0/800 | 1,600/0/800 | - | - | NP | NP | - | Endocarditis |
| 14 | 1,600/50/25 | 3,200/100/50 | NP | NP | NP | NP | - | Endocarditis |
| 15 | 12,800/50/3,200 | 25,600/100/6,400 | - | NP | NP | NP | - | Endocarditis |
| 16 | 102,400/0/0 | 204,800/0/0 | NP | NP | + | + | - | Vascular infection |
| 17 | 51,200/50/25 | 102,400/100/50 | NP | - | NP | NP | - | Endocarditis |
| 18 | 6,400/0/800 | 12,800/0/1,600 | NP | + | + | + | - | Endocarditis |
| 19 | 6,400/50/100 | 25,600/100/400 | NP | NP | + | + | - | Endocarditis |
| 20 | 12,800/0/1,600 | 25,600/0/1,600 | NP | NP | NP | NP | - | Endocarditis |
| 21 | 1,600/0/100 | 6,400/0/200 | NP | NP | NP | NP | - | Endocarditis |
| 22 | 25,600/800/6,400 | 102,400/800/12,800 | NP | NP | NP | NP | - | Endocarditis |
| 23 | 6,400/100/400 | 25,600/1,600/1,600 | NP | NP | + | + | - | Endocarditis |
| 24 | 25,600/100/0 | 102,400/200/0 | NP | NP | NP | NP | - | Endocarditis |
| 25 | 1,600/0/0 | 1,600/50/0 | NP | NP | NP | NP | + | Endocarditis |
| 26 | 3,200/3,200/100 | 25,600/12,800/200 | - | NP | NP | NP | - | Endocarditis |
| 27 | 1,600/0/200 | 3,200/0/200 | NP | NP | NP | NP | - | Endocarditis |
| 28 | 51,200/200/3,200 | 51,200/200/12,800 | NP | NP | NP | NP | - | Endocarditis |
| 29 | 3200/400/0 | 6,400/800/0 | NP | NP | NP | NP | - | Endocarditis |
| 30 | 6,400/1,600/0 | 1,600/400/0 | NP | - | NP | NP | - | Endocarditis |
| 31 | 12,800/0/25 | 25,600/25/50 | NP | NP | NP | NP | + | Endocarditis |
| 32 | 25,600/200/800 | 51,200/400/1,600 | NP | NP | NP | NP | - | Endocarditis |
| 33 | 12,800/0/400 | 25,600/0/800 | NP | NP | NP | NP | - | Endocarditis |
| 34 | 12,800/400/800 | 51,200/800/1,600 | NP | NP | NP | NP | - | Endocarditis |
| 35 | 25,600/50/25 | 51,200/100/50 | NP | NP | NP | NP | - | Endocarditis |
| 36 | 3,200/25/1,600 | 1,600/25/1,600 | NP | NP | NP | NP | - | Endocarditis |
| 37 | 6,400/800/50 | 3,200/800/25 | NP | NP | NP | NP | - | Endocarditis |
| 38 | 3,200/50/100 | 25,600/100/200 | NP | NP | NP | NP | + | Endocarditis |
| 39 | 1,600/0/0 | 3,200/0/0 | NP | NP | NP | NP | + | Endocarditis |
| 40 | 800/100/100 | 3,200/200/200 | NP | NP | NP | NP | + | Vascular infection |
| 41 | 800/0/0 | 800/0/25 | NP | NP | + | + | + | Endocarditis |
| 42 | 6,400/50/1,600 | 12,800/100/3,200 | NP | NP | NP | NP | + | Endocarditis |
| 43 | 3,200/100/25 | 12,800/200/25 | NP | NP | NP | NP | + | Endocarditis |
| 44 | 25,600/0/6,400 | 51,200/0/6,400 | NP | NP | - | - | - | Vascular infection |
| 45 | 25,600/50/400 | 51,200/50/800 | NP | NP | NP | NP | - | Endocarditis |
| 46 | 3,200/50/0 | 6,400/50/0 | NP | NP | NP | NP | + | Endocarditis |
| 47 | 25,600/0/400 | 51,200/0/800 | NP | NP | + | + | - | Endocarditis |
| 48 | 102,400/25/12,800 | 204,800/25/25,600 | NP | NP | + | + | - | Endocarditis |

^a Values for analyses of sera from patients 1 to 37 were retrospectively analyzed; sera from patients 38 to 48 were prospectively analyzed. NP, not performed. MIF, microimmunofluorescence.

action of the RF-Absorbent reagent, the production of pathogen-specific immune complexes during the subsequent test is inhibited.

Statistical analysis. To estimate the influence of the delay of sample conservation and serum sampling on the sensitivity of the LCN-PCR, we compared the mean age of serum specimens with the Fischer's test with Epi Info, version 6.04a (Centers for Disease Control and Prevention, Atlanta, Ga.). *P* values of <0.05 were considered statistically different.

RESULTS

Patients' characteristics. All the data are summarized in Tables 2 and 3. Among the group of sera stored and retrospectively analyzed, 28 of 37 patients were male. Their mean age

was 62.3 ± 12.6 years (range, 42 to 80 years). Thirty-five patients presented with endocarditis, and 2 had vascular infections. Among the group of patients from which fresh serum samples were prospectively analyzed, 8 of 11 patients were male. Their mean age was 59.8 ± 18.4 years (range, 15 to 80 years). Nine patients presented with endocarditis, and 2 had vascular infections.

Serology results. All the data for serology results are summarized in Table 3. Among the group of stored sera, the IgG phase I titer was less than 1:25,600 for 26 patients and equal to or greater than 1:25,600 for 11 patients. Among the group of

TABLE 3. Influence of serum storage and antibodies on the LCN-PCR results

| Serum sample | No. of patients tested | Mean patient age (yr) | No. of positive samples | | |
|----------------------|------------------------|-----------------------|-----------------------------------|-----------------------------------|------------------------------------|
| | | | Irrespective of IgG phase I titer | IgG phase I titer $\geq 1:25,600$ | No. IgG phase I titer $< 1:25,600$ |
| Stored | 37 | 62.3 | 6/37 | 0/11 | 6/26 |
| Prospectively tested | 11 | 59.8 | 7/11 | 0/4 | 7/7 |
| Total | 48 | 61.8 | 13/48 | 0/15 | 12/33 |

fresh serum samples directly analyzed, the IgG phase I titer was less than 1:25,600 for seven patients and equal to or greater than 1:25,600 for four patients. When all serum sample results were considered, the IgG phase I titer ranged from 1:800 to 1:102,400 with a mean of $1:18,873 \pm 26,773$.

LCN-PCR. The data are summarized in Tables 2 and 3. The overall sensitivity of LCN-PCR was 27% (13 of 48 samples). When comparing patients with respect to the delay between serum sampling and completion of LCN-PCR, sera conserved more than 1 month exhibited fewer positive results (6 of 37 samples; 16.2%) than samples prospectively and directly analyzed (7 of 11 samples; 64%). This difference was statistically significant ($P = 0.004$).

When estimating the role of IgG phase I titer on the sensitivity of LCN-PCR among all sera, a significant difference ($P = 0.004$) was observed among LCN-PCR results for sera with phase I IgG titers equal to or greater than 1:25,600 (0 of 15 samples; 0%) compared to sera with phase I IgG titers less than 1:25,600 (13 of 33 samples; 39.4%). It is also important to underline that among the fresh serum samples analyzed directly, the sensitivity of the test was 100% (7 of 7) among samples with antibodies titers less than 1:25,600. LCN-PCR also exhibited a specificity of 100%. All the sera from the 30 patients treated for Q fever endocarditis and from the 13 patients with a diagnosis of acute Q fever for whom the IgG phase I titer was equal to or greater than 1:800 were negative by the LCN-PCR. In addition, all the samples from our control group of patients with endocarditis caused by other microorganisms were also LCN-PCR negative. For the 12 patients for whom the first serum sample was positive by LCN-PCR, the second sera sampled after 1 month of therapy was LCN-PCR negative for all the tested sera. For the four fresh sera with IgG phase I titers equal to or greater than 1:25,600, the LCN-PCR results were still negative after the IgG was removed, thereby inhibiting the production of pathogen-specific immune complexes.

It is important to underline that the positivity of all PCR products was confirmed by sequences identical to that of *C. burnetii*. Indeed, as LCN-PCR is a nested PCR, several amplicons can be obtained. The interpretation of positivity may then be difficult. Thus, it is necessary when several PCR products are present, including products of the expected size, that sequencing is performed to confirm the positivity of the test. An agarose gel profile of PCR products is presented in Fig. 1.

DISCUSSION

In the present study, we report the adaptation of the LCN-PCR assay to the diagnosis of Q fever endocarditis and vascular infections. Chronic Q fever often presents with unspecific

symptomatology and few symptoms. Without adequate antibiotic therapy, the natural evolution of this disease is death. Given the potential severity of chronic Q fever and the efficiency of oral antibiotics, it is particularly important to perform the diagnosis as early as possible. However, the diagnosis of chronic Q fever is still difficult. First, physicians do not always systematically consider this microorganism as a cause of endocarditis or vascular infections. Second, it is difficult to isolate *C. burnetii* not only from blood samples but also from biopsy samples, such as cardiac valves or vascular specimens, which also requires invasive procedures. Indeed, cell culture competence and biosafety level 3 laboratory conditions are required for the culture of this bacterium (24). Third, the commercialized kits available for the analysis of Q fever serology do not usually permit the investigation of phase I antigens; subsequently, the diagnosis of chronic Q fever cannot be achieved. Indeed, the production and the conservation of phase I antigen is difficult, requiring competence at performing animal inoculation in a biosafety level 3 laboratory setting (14). Thus, only reference laboratories for *C. burnetii* could propose an accurate diagnosis of chronic Q fever, but a problem of interpretation is still present when the IgG phase I titer is equal to 1:800.

Serum is one of the easiest human samples to obtain. Also, when sampled early in the evolution of a systemic disease, it is likely to contain DNA copies of the involved pathogens (21). Thus, it could be a useful sample for direct diagnosis. Due to the presence of inhibitors in blood (1, 3) and to the small amount of bacterial DNA present in serum, typical PCR assays are not efficient. Presently, the availability of bacterial genome data allows a best choice of DNA targets for PCR assays. It has previously been demonstrated that the targeting of repeated multicopy sequence increases the sensitivity of the PCR assay without altering its specificity (11, 30). Toward this end, PCR assays targeting the *htpAB*-associated repetitive element,

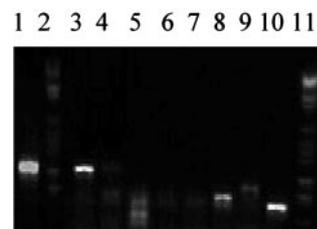


FIG. 1. Agarose gel electrophoresis of *Coxiella burnetii* LCN-PCR. Lanes: 1, *C. burnetii* DNA control (band represents expected 260-bp PCR product); 2 and 11, molecular size marker; 3 and 4, samples from patients with an evolutive Q fever endocarditis; 5 to 7, samples from patients with a convalescent Q fever; 8 to 10, samples from patients treated for a Q fever endocarditis for more than 1 month.

which exists in 20 copies in the genome of *C. burnetii*, have been successfully used (30). Complete genome sequencing of Nine Mile strains confirmed that this is the most frequently repeated sequence (25). Nested-PCR approaches were shown also to be more sensitive but could present less specificity, due to PCR contamination resulting from lateral contamination during the amplification (31). For the diagnosis of *Bartonella* endocarditis, a sensitive nested-PCR assay has been successfully performed with the LightCycler which offers the advantage, in avoiding contamination, of not requiring the reaction tubes to be opened during the amplification process (31).

A combination of these two approaches, based on a LCN-PCR assay targeting a repetitive element sequence, has been previously reported for the diagnosis of acute Q fever (15). This prospective study demonstrated that the LCN-PCR is useful to carry out serology for the early diagnosis of acute Q fever with a sensitivity of 26% when no antibodies were detected but a sensitivity of only 5% for seropositive patients. It was positive only in when samples from patients with evolutive acute Q fever were analyzed.

For chronic Q fever, an overall sensitivity of 26% is observed with this new tool, but the test sensitivity significantly increases (64%) when the analysis was performed prospectively with fresh sera. Thus, the sensitivity of our LCN-PCR assay was strongly affected by serum storage at -20°C in samples from patients with chronic Q fever, most likely through the progressive degradation of frozen DNA. Apparently, degradation was more rapid than for samples from patients with acute Q fever; this may be related to the presence of elevated antibody titers. Also, this low level of LCN-PCR sensitivity with stored sera was previously noticed for the diagnosis of *Bartonella* endocarditis, but the evidence was even stronger with samples from patients with chronic Q fever. Indeed, for *Bartonella* endocarditis, a sensitivity of 85.7% was noticed with sera stored for less than 1 year, and the sensitivity progressively decreased to 62.5, 53.3, and 46.1% for tests performed on sera stored for 1 to 3 years, 3 to 5 years, and more than 5 years (31). In view of the alteration of stored DNA at -20°C , it will be interesting in the future to store the sera at -80°C when performing LCN-PCR after various lengths of storage to verify if DNA is conserved in these conditions.

Interestingly, the sensitivity of our LCN-PCR assay was significantly altered with very high IgG phase I antibody titers. In contrast, for samples from patients with *Bartonella* endocarditis, the sensitivity of LCN-PCR was not altered despite elevated antibody titers. We could not clearly explain this phenomenon; only hypotheses could be suggested. Elevated IgG phase I titers may determine the production of pathogen-specific immune complexes, leading to a negative LCN-PCR result. However, when the IgG was removed, the LCN-PCR results were still negative. The immune complexes may generate cryoglobulins, and it will be interesting to search for the presence of cryoglobulins and to perform LCN PCR with the cryoprecipitate. It is very important to note that in chronic Q fever, very high levels of antibody titers are usually observed in comparison to other infective endocarditis. For example, a mean of IgG phase I titer of 1:16,823 was observed in this study, compared to a mean of IgG titer of 1:4,251 in a previous study of *Bartonella* endocarditis by LCN-PCR (31). IgG has been described as an inhibitor of diagnostic PCR assays (2).

Finally, it is important to take in consideration that an IgG phase I titers equal to or greater than 1:25,600 is an unambiguous biological sign of chronic Q fever. The main problem is to establish a certain diagnosis of chronic Q fever in patients with IgG phase I titers ranging from 1:800 to 1:1,600. In these cases, LCN-PCR will be a clue for establishing an accurate diagnosis of chronic Q fever. Additionally, for these patients, LCN-PCR seems to be a useful tool for therapeutic follow-up. Finally, LCN-PCR represents a good predictive value for the diagnosis of evolutive Q fever. In cases of acute Q fever, when the antibodies increased, the LCN-PCR became negative. However, in cases of evolution to a chronic Q fever, when the antibodies increased, the LCN-PCR was positive and became negative only when the IgG phase I titer was very high (i.e., equal to or greater than 1:25,600) or when an efficient treatment had been underway for 1 month.

In conclusion, the LCN-PCR assay seems to be a valuable tool for the diagnosis of Q fever endocarditis and vascular infection, when applied to fresh sera. This technique may help shorten the delay of diagnosis of these infections, mainly in patients with IgG phase I titers ranging from 1:800 to 1:6,400. This assay may also be used for follow-up to test the efficiency of the treatment.

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