INTRODUCTION

Bartonella quintana is a Gram-negative bacillus belonging to the α2 subgroup of Proteobacteria. The first recognized clinical manifestation of this louse-borne pathogen was trench fever, described first in Europe during World War I and then re-emerging during World War II, resulting in large-scale epidemics. The infection is ubiquitous and has been described in Japan, Peru, Mexico, and Burundi. Later, B. quintana was recognized as an etiologic agent of cutaneous bacillary angiomatosis, bacteremia, infective endocarditis (IE), and chronic lymphadenopathy in patients infected with HIV. In the past decade, B. quintana has gained importance as an agent of blood culture negative endocarditis (BCNE) in immunocompetent patients. Since then, many cases of endocarditis due to B. quintana and other species of the genus Bartonella have been reported. They are thought to account for 1% to 3% of all endocarditis. Because Bartonella endocarditis seems to be very common in Tunisia, we suggest that its serology be performed systematically whenever endocarditis is suspected.

PATIENTS AND METHODS

Patients. Sera from patients suspected to have endocarditis were collected at the CHU Habib Bourguiba hospital of Sfax from January 1997 to December 2003. IE was diagnosed using the modified Duke criteria.

Serology. Screening: sera were screened for Chlamydia pneumoniae, C. psittaci, and C. trachomatis in an MIF assay using antigens prepared in house from embryonic eggs. IgG titers of 1:64 or above were considered positive. Sera were also tested using C. burnetii phase II antigens (BioMérieux, Marcy L’Etoile, France). The cutoff for IgG was 1:200. Sera positive either for Chlamydia species or C. burnetii were stored at −80°C until their use in the “Unité des Rickettsies” in Marseille, France.

Serology for Bartonella spp. and C. burnetii was performed using a MIF assay as previously described. The cutoff value to diagnose endocarditis was 1:800 for both IgG against phase I C. burnetii and Bartonella spp.

Western immunoblotting was performed on samples positive for Bartonella as previously reported. Western blot analysis was performed both before and after cross-absorption with B. quintana and B. henselae antigens.

For one patient, diagnosis of chlamydial endocarditis was made previously by PCR on valve tissue. For this patient, a Western blot with C. pneumoniae antigens provided by MicroBix (Biosystems Inc., Ontario, Canada) before and after cross-absorption with either C. pneumoniae or B. quintana was performed.

Molecular biology. DNA extraction was performed from sera with a QIAamp blood kit (Qiagen, Hilden, Germany). We performed two light cycler nested PCRs (LCN) in one step according to our previous report, using external and internal primers amplifying rnpb gene and fur gene. Sequences of primers are shown in Table 1. A negative control of serum was added every three samples. The positive control was Bartonella elizabethae.

Purified PCR products were sequenced using an ABI 3100 automated sequencer (Perkin-Elmer, Cogniers, France). Nucleotide sequences were edited with the Bioedit package (version 7.2.5; Ibisys, St Neots, UK) and multiple alignments with Bartonella spp. sequences available from GenBank were carried out using the CLUSTAL W software, version 1.81.

RESULTS

At screening, 40 sera were positive against Chlamydia spp. antigens whereas one serum was positive for C. burnetii, Us-
ing specific MIF assay in the French national reference center, the patient with positive serology for \textit{C. burnetii} was shown to have phase I IgG titers at 1:1600, thus confirming the diagnosis of Q fever endocarditis.\textsuperscript{21} Among the 40 patients with positive results for \textit{Chlamydia} spp., 13 were positive with \textit{Bartonella} spp. with IgG titers > 1:800 but were negative for \textit{C. burnetii} antibodies (Table 2). Western blot was able to confirm diagnosis of \textit{B. quintana} in 12 cases (92\%) and \textit{B. henselae} in one case. Of note, none of the remaining 27 \textit{Chlamydia} positive and \textit{Bartonella} negative sera were sampled from patients with IE.

PCR results are shown in Table 2. LCN PCR amplifying \textit{rmpb} gene was positive in 58.3\% whilst LCN PCR targeting the \textit{fur} gene in 92.3\% Among the 13 patients with positive \textit{Bartonella} serology, the diagnosis of definite endocarditis according to Duke criteria was made in 11 cases. During the period of the study, 112 definite diagnoses of IE were made in the cardiology department at CHU of Sfax, 51 (45.5\%) of which were classified as BCNE. Therefore, these 11 cases of \textit{B. quintana} endocarditis accounted for at least 9.8\% of all definite IE. For the remaining two patients (nos. 10 and 13), lack of cardiac echography did not allow us to confirm the diagnosis. These two cases were considered possible diagnosis of IE. Interestingly, for patient no. 4 a diagnosis of endocarditis due to \textit{C. pneumoniae} was previously made via PCR of 16S rRNA gene on cardiac valve tissue and by \textit{in situ} hybridization.\textsuperscript{15} For this patient, LCN PCR targeting \textit{rmpb} gene on serum was negative but LCN PCR targeting \textit{fur} gene was positive. The sequence amplified was identical to \textit{B. quintana} with a percentage homology of 98\%. Furthermore, the western blot with cross absorption for this patient showed an infection with \textit{B. quintana}. Epidemiologic data was available for 12 patients. The mean age of patients with \textit{Bartonella} IE (possible or definite) was 39.58 ± 12.55 years with a range of 20 to 56 years. Sex ratio M/F was 3.33 with 10 (76.9\%) men and 3 (23.1\%) women. Eleven patients (92\%) were of rural origin with poor living conditions. Eight patients had contact with animals (66.6\%). Seven patients (58\%) had a history of valvulopathy whilst the patient with \textit{B. henselae} had a prosthetic valve. One patient was immunocompromised; he had drug induced lupus and was undergoing treatment with corticosteroids. Seven (58\%) patients presented with fever. One patient first presented with renal insufficiency and had extra-capillary glomerulonephritis. Five cases had mitral valve involvement, two had aortic valve involvement, four had mid-nenent a both valves and for the last case there is no data. All patients but three had received \beta-lactam and aminoglycoside therapy. The patient who did not receive antibiotics had ischemic cerebral vascular accident and died before commencing treatment. Seven (58\%) patients underwent valvular re-

### Table 1

Sequence of primers used in LCN PCR

<table>
<thead>
<tr>
<th>Primers of \textit{rmpb} gene</th>
<th>Sequences</th>
<th>Length of amplified fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>External primers forward</td>
<td>5'-GRTCCGGGAGGAAAGTC-3'</td>
<td>256 pb</td>
</tr>
<tr>
<td>External primers reverse</td>
<td>5'-GGAAAAACGRTGCCGGGTA-3'</td>
<td>263 pb</td>
</tr>
<tr>
<td>Internal primers forward</td>
<td>5'-AACCTACCRAATGACTG-3'</td>
<td>190 pb</td>
</tr>
<tr>
<td>Internal primers reverse</td>
<td>5'-CCATTYCATCTGGACGGAT-3'</td>
<td>175 pb</td>
</tr>
</tbody>
</table>

### Table 2

Results of serology, Western blot, PCR, antibiotic therapy, and outcome of patients with \textit{B. quintana} endocarditis from cardiology service of CHU of Sfax, Tunisia

<table>
<thead>
<tr>
<th>Case/sex/age</th>
<th>Serology</th>
<th>LCN PCR</th>
<th>Final diagnosis</th>
<th>Antibiotic therapy</th>
<th>Operated</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/44</td>
<td>+</td>
<td>+</td>
<td>6400</td>
<td>6400</td>
<td>BQ</td>
<td>BQ</td>
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<tr>
<td>2/F*</td>
<td>+</td>
<td>+</td>
<td>1600</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>3/M/46</td>
<td>+</td>
<td>+</td>
<td>1600</td>
<td>1600</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>4/F/56</td>
<td>+</td>
<td>+</td>
<td>6400</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>5/M/31</td>
<td>+</td>
<td>+</td>
<td>3200</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>6/M/44</td>
<td>+</td>
<td>+</td>
<td>6400</td>
<td>6400</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>7/M/48</td>
<td>+</td>
<td>+</td>
<td>3200</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>8/F/26</td>
<td>+</td>
<td>+</td>
<td>3200</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>9/M/44</td>
<td>+</td>
<td>+</td>
<td>3200</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>10/M/56</td>
<td>+</td>
<td>+</td>
<td>800</td>
<td>400</td>
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<tr>
<td>11/M/40</td>
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<td>+</td>
<td>6400</td>
<td>3200</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>12/M/20</td>
<td>+</td>
<td>+</td>
<td>3200</td>
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<td>BQ</td>
<td>BQ</td>
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<tr>
<td>13/M/20</td>
<td>+</td>
<td>+</td>
<td>1600</td>
<td>1600</td>
<td>BQ</td>
<td>BQ</td>
</tr>
</tbody>
</table>

**Note:**
- For patients 2 and 13, no data were available.
- For patients 10 and 13, no data were available.
- Antibiotics: amik, amicacin; amphotericin B; Ampi, ampicillin; doxy, doxycyclin; gena, gentamicin; oflo, ofloxacin; Peni G, penicillin G; vanco, vancomycin; sxt, cotrimoxazole. BH, \textit{B. henselae} | BQ, \textit{B. quintana}; Ch, \textit{Coxiella burnetii}. LS, lost of sight; WB, Western blot.
- †Diagnosis of endocarditis due to \textit{C. pneumoniae} was made by PCR performed on the valve.
placement and recovered. Unfortunately, valves removed from these patients were not stored. One patient did not undergo surgery and recovered.

**DISCUSSION**

In this study, using a variety of techniques, we have confirmed *Bartonella* IE in patients in Tunisia. These methods have been previously reported to be both specific and sensitive.\(^9,10,17,18\) Using MIF, all 13 patients had titers \(\geq 1:800\). Such high titers have a positive predictive value of 95.5% for endocarditis.\(^9,10\) Western blot with cross-absorption was used to discriminate between species.\(^12\) Moreover, LCN PCR amplifying *rnpb* gene was positive in 7 cases (58.3%), this percentage was similar to our previous report in which sensitivity of LCN PCR with *ribC* gene was 58.1%.\(^18\) The LCN PCR on sera targeting *fur* gene increased the sensitivity of the results; it was positive in 12 cases (92.3%). This is somewhat surprising because the two LCN PCR target genes have only one copy in the *Bartonella* genome.\(^20,21\) Our study reemphasizes the fact that the existence of cross-reactions between *Bartonella* and *Chlamydia* can lead to wrong diagnosis. Patients with *B. quintana* endocarditis were shown to display cross-reacting antibodies to *C. pneumoniae*, *C. psittaci*,\(^5\) and *C. burnetii*.\(^11\) It has been previously demonstrated that almost all cases of *Chlamydia* endocarditis were in fact *Bartonella* endocarditis.\(^15\) The major *Chlamydia*-cross-reacting protein antigen was about 60 to 70 kDa, which may correspond to a heat shock protein. Although *Bartonella* endocarditis has been well characterized by culture and/or PCR from valves, diagnosis of *Chlamydia* endocarditis was often based solely on serology. There is only one report of the presence of *Chlamydia pneumoniae* in cardiac valve by PCR and in situ hybridization.\(^15\) Interestingly the case of this patient corresponds to the patient no. 4 in our series. However, serology, western blot and PCR against *Bartonella* were not performed for the patient in this report.\(^15\) Using Western blot, we have shown that this patient had in fact *Bartonella* endocarditis. The presence of *C. pneumoniae* in his cardiac valve could be explained by the fact that this bacterium has a tropism for the human vascular system. It has especially been associated with cardiovascular diseases and detected in atherosclerotic plaques.\(^22\)

Twelve (92%) of our cases were due to *B. quintana*, *B. henselae* was found in only one case. In the literature *B. quintana* accounts for three quarter of *Bartonella* endocarditis.\(^8\) *B. quintana* endocarditis has been associated with alcoholism, homelessness and body lice.\(^8\) In our study, no patient was homeless or an alcoholic and none had evidence of body lice. However, 11 (92%) of the infected were from rural areas and 8 (66.6%) had contact with animals. Although the only known reservoir of *B. quintana* is human\(^23\) the bacteria has recently been demonstrated in fleas.\(^24\) These may therefore play a role in the transmission of the bacteria to humans from a non human reservoir. A study performed in rodents, *Psammomys obesus*, in Tunisia showed that 49% were infected with *Bartonella* species with a recrudescence in August and September.\(^25\)

In our series, patients treated with aminoglycosides and \(\beta\)-lactam antibiotics were cured. However, valve replacement was necessary in the majority of cases due to extensive valvular damage as previously reported.\(^26\) We have previously

![Figure 1](image.png)

**Figure 1.** Proportion of *Bartonella* endocarditis among IE in Europe and North Africa.
Recommended that effective antibiotic therapy for Bartonella endocarditis should include an aminoglycoside for at least 14 days to achieve a bactericidal effect.26,27 Patient no. 7, who did not receive antibiotics, died.

In our study, BCNE accounted for 45% of IE. The incidence of BCNE may vary considerably from 1 to 79%.28 It is reported to be higher in developing countries (53 to 79% in India, 47.5% in south Africa and 48% in Pakistan).29,30 The main cause of BCNE is previous antibiotic therapy. Many etiological agents have been incriminated such as HACEK group bacteria, Bartonella, C. burnetii, and Tropheryma whipplei.31 Several studies of Bartonella endocarditis have previously been published with percentages ranging from 0 to 4.5% of all IE.7,31–33 In our series, we found a higher prevalence of Bartonella endocarditis (at least 9.8%), which is probably due to differences in living conditions or higher temperature, but in any way to homelessness. Data about body louse infection in our patients was lacking (Figure 1).

In conclusion, B. quintana appears to be a frequent agent of BCNE in Tunisia. Therefore specific serology should be performed systematically whenever diagnosis of IE is suspected. If left untreated, Bartonella endocarditis can be fatal. The treatment recommended for these infections should include aminoglycosides for at least 2 weeks in association with amoxicillin for 6 weeks.26,27 However, more prospective studies are needed to draw conclusions with regard to epidemiologic characteristics and clinical aspects of this infection.

Received August 26, 2004. Accepted for publication November 6, 2004.

Acknowledgments: The authors thank Hachicha Sajiaa for his assistance to perform this work and E. Mathai for reviewing the manuscript. Among potential conflicts of interest, D. Raoult has deposited a patent on serological diagnosis of infective endocarditis, mainly based on the article: Rolain JM, Lecam C, Raoult D. Simplified serological diagnosis of endocarditis due to C. burnetii and Bartonella. Clin Diag Lab Immunol 2005;12(6):1147–1148.

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