Bartonella vinsonii subsp. arupensis as an Agent of Blood Culture-Negative Endocarditis in a Human

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We report the case of a patient hospitalized with endocarditis. The etiological diagnosis of Bartonella was suggested by detection of high titers of antibodies by immunofluorescence and Western blotting. Two different nested PCRs performed on sera identified Bartonella vinsonii subsp. arupensis by sequencing.

There are now 19 species within the genus Bartonella (11). Eight have been implicated in human diseases. Among these, four have been recognized as agents of infective endocarditis (IE): Bartonella henselae, Bartonella quintana, and, in one case each, Bartonella elizabethae and Bartonella vinsonii subsp. berkholffii (1, 8, 9). Bartonella endocarditis represents 3% of all cases of endocarditis in France. Serology, mainly the indirect immunofluorescence antibody (IFA) assay, is an excellent tool for its diagnosis (7), but cross-reactivity among Bartonella spp. has been observed (4). Western blotting allows a specific diagnosis when performed with adsorbed sera (4). Recently, a nested PCR was proposed as a diagnostic tool (11). We routinely perform Western blotting and PCR on sera from patients with suspected Bartonella endocarditis. Using these techniques, we have diagnosed a case of endocarditis due to Bartonella vinsonii subsp. arupensis.

A 79-year-old male was admitted to the hospital in June 2003 after having felt unwell for a month. He had received an aortic valve bioprosthesis in 1999. The retired man lived in an urban setting and had no history of exposure to animals. At admission, he presented with a fever of 38°C. Auscultation of the aortic valve bioprosthesis in 1999. The retired man lived in an urban setting and had no history of exposure to animals. At admission, he presented with a fever of 38°C. Auscultation of the aortic valve bioprosthesis showed a new regurgitation no. AF410937). To confirm this finding, we performed a second nested PCR targeting a 50-bp GroEL sequence using PCR performed using B23SF1 (5′-GGGTTCCTGCTTAAAGTT-3′) and B23SR1 (5′-CGCACAGCCTGTTGTGTTT-3′) as external primers and B23Sseq2 (5′-CAGCCTTCATCCGAAATT-3′) and B23Sfseq2 (5′-TATTCTGAGCAGGTGA-3′) as internal primers. A PCR product was detected and sequenced, as previously described (11). The sequence obtained was 100% similar to that of Bartonella vinsonii subsp. arupensis (GenBank accession no. AF410937). To confirm this finding, we performed a second nested PCR targeting a 50-bp GroEL sequence using

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GroEL-1F (5'-CACAAATGCTGAGAAAATGG-3') and GroEL-1R (5'-CATATCCAAAGTGACATTTC-3') as external primers and GroEL-2F (5'-CCACTTCTTATTATCGC-3') and GroEL-2R (5'-ATTTTCAAACCACCACG-3') as internal primers. A PCR product was obtained and sequenced. The sequence obtained was 100% similar to that of *B. vinsonii* subsp. *arupensis* (GenBank accession no. AF304016.1). For both nested PCRs, all the negative controls, which included a mixture of all reagents used for DNA extraction and DNA extracted from normal heart tissue, were PCR negative.

IE is a life-threatening disease. It is critical that a diagnosis be made as early as possible. Here, we confirm that IFA serology is a useful tool for the diagnosis of *Bartonella* endocarditis when high titers (≥1:400) are found (3, 7). A positive diagnosis of a *Bartonella* sp. can be made even when the *Bartonella* sp. involved is not tested in the assay due to antigen cross-reactivity. Western blotting performed after adsorption allowed a specific diagnosis of the species *B. vinsonii* but did not distinguish among subspecies. The results obtained by Western blotting were confirmed by those obtained by using two nested PCRs. The first nested PCR was highly specific, because the targeted sequence was used for the first time in the laboratory, with no possible risk of contamination with previous amplified DNA (we call it “suicide PCR”) (6). Based on our findings, *B. vinsonii* subsp. *arupensis* should be added to the list of pathogens capable of causing endocarditis.

### TABLE 1. Results of serological analysis performed on five serum samples and PCR assays performed on the first two serum samples of the patient

<table>
<thead>
<tr>
<th>Serum sampling date</th>
<th>IgG titer against antigens of:</th>
<th>PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>B. henselae</em></td>
<td><em>B. quintana</em></td>
</tr>
<tr>
<td>5 June&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1:400</td>
<td>1:400</td>
</tr>
<tr>
<td>25 June&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:400</td>
<td>1:200</td>
</tr>
<tr>
<td>30 June&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:200</td>
<td>1:100</td>
</tr>
<tr>
<td>31 July&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:100</td>
<td>1:50</td>
</tr>
<tr>
<td>2 September&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1:50</td>
<td>1:50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sample was taken before therapy.

<sup>b</sup> PCRs targeting the 23S rRNA and GroEL DNA sequences were positive and allowed the identification of *B. vinsonii* subsp. *arupensis*.

<sup>c</sup> Sample was taken during therapy.

<sup>d</sup> ND, not done.

<sup>e</sup> Sample was taken after therapy.

![Western blotting](http://jcm.asm.org/)

**FIG. 1.** Western blotting performed with the first serum sample from 5 June at a 1:200 dilution. Molecular masses (in kilodaltons) are given on the left. (A through E) Serum was analyzed by using *B. quintana* (lane 1), *B. henselae* (lane 2), *B. elizabethae* (lane 3), *B. vinsonii* subsp. *arupensis* (lane 4), and *B. vinsonii* subsp. *berkhoffii* (lane 5) antigens. (A) Untreated serum. (B) *B. vinsonii* subsp. *arupensis*-adsorbed serum. All antibodies were removed. (C) *B. vinsonii* subsp. *berkhoffii*-adsorbed serum. All antibodies were removed. (D) *B. quintana*-adsorbed serum. Antibodies to the two subspecies of *B. vinsonii* remained. (E) *B. henselae*-adsorbed serum. Antibodies to the two subspecies of *B. vinsonii* remained. (F through H) Serum was analyzed by using *B. vinsonii* subsp. *arupensis* (lanes 4) and *C. pneumoniae* (lane 6) antigens. (F) Untreated serum. Note the lower reaction to *C. pneumoniae*. (G) *B. vinsonii* subsp. *arupensis*-adsorbed serum. All antibodies were removed. (H) *C. pneumoniae*-adsorbed serum. Antibodies to *B. vinsonii* subsp. *arupensis* remained.
in humans. In 1998, the DNA of this bacterium was detected incidentally for the first time during the course of studies on the reservoirs of tick-borne pathogens in Minnesota and Wisconsin when amplification of *Bartonella*-like 16S rRNA segments was observed in the blood of mice (10). One year later, this bacterium was isolated in Wyoming from the blood culture of a cattle rancher with valvulopathy, but the exact contribution of this *Bartonella* isolate to the patient’s illness was not clear (10). The role of *Bartonella* in patients with blood culture-negative endocarditis could be supported by a combination of other factors, such as contact with body lice and unknown valvulopathy for *B. quintana*, or contact with cats and known valvulopathy for *B. henselae*. For *B. vinsonii* subsp. *arupensis*, a rodent reservoir host has been suggested. Our patient could not remember being in contact with rodents, and he presented with known valvular damage.

Finally, our data also underline the fact that cross-reactions between *Bartonella* and *Chlamydia* spp. occur (2, 5) and can lead to misdiagnoses of *Chlamydia* endocarditis. Indeed, it has been shown that almost all cases of *Chlamydia* endocarditis were in fact *Bartonella* endocarditis (5). Here, we show that *B. vinsonii* subsp. *arupensis* is a human pathogen responsible for endocarditis.

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REFERENCES


AUTHOR’S CORRECTION

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