Culture of *Bartonella quintana* and *Bartonella henselae* from Human Samples: a 5-Year Experience (1993 to 1998)

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*Bartonella quintana* and *Bartonella henselae* are fastidious gram-negative bacteria responsible for bacillary angiomatosis, trench fever, cat scratch disease, and endocarditis. During a 5-year period, we received 2,043 samples for culture of *Bartonella* sp. We found *Bartonella* sp. to be the etiologic agent in 38 cases of endocarditis, 78 cases of cat scratch disease, 16 cases of bacteremia in homeless people, and 7 cases of bacillary angiomatosis. We correlated the results of positive cultures with the clinical form of the disease, type of sample, culture procedure, PCR-based genomic detection, and antibody determination. Seventy-two isolates of *B. quintana* and nine isolates of *B. henselae* from 43 patients were obtained. Sixty-three of the *B. quintana* isolates and two of the *B. henselae* isolates, obtained from patients with no prior antibiotic therapy, were stably subcultured. The sensitivity of culture was low when compared with that of PCR-based detection methods in valves of patients with endocarditis (44 and 81%, respectively), skin biopsy samples of patients with bacillary angiomatosis (43 and 100%, respectively), and lymph nodes of cat scratch disease (13 and 30%, respectively). Serological diagnosis was also more sensitive in cases of endocarditis (97%) and cat scratch disease (90%). Among endocarditis patients, the sensitivity of the shell vial culture assay was 28% when inoculated with blood samples and 44% when inoculated with valvular biopsy samples, and the sensitivity of both was significantly higher than that of culture on agar (5% for blood [P = 0.045] and 4% for valve biopsy samples [P < 0.0005]). The most efficient culture procedure was the subculture of blood culture broth into shell vials (sensitivity, 71%). For patients with endocarditis, previous antibiotic therapy significantly affected results of blood culture; no patient who had been administered antibiotics yielded a positive blood culture, whereas 80% of patients with no previous antibiotic therapy yielded positive blood cultures (P = 0.0006). Previous antibiotic therapy did not, however, prevent isolation of *Bartonella* sp. from cardiac valves but did prevent the establishment of strains, as none of the 15 isolates from treated patients could be successfully subcultured. For the diagnosis of *B. quintana* bacteremia in homeless people, the efficiency of systematic subculture of blood culture broth onto agar was higher than that of direct blood plating (respective sensitivities, 98 and 10% [P < 10⁻⁴]). Nevertheless, both procedures are complementary, since when used together their sensitivity reached 100%. All homeless people with positive blood cultures had negative serology. The isolation rate of *B. henselae* from PCR-positive lymph nodes, in patients with cat scratch disease, was significantly lower than that from valves of endocarditis patients and skin biopsy samples from bacillary angiomatosis patients (13 and 33%, respectively [P = 0.084]).

In cases of bacillary angiomatosis for which an agent was identified to species level, the isolation rate of *B. henselae* was lower than the isolation rate of *B. quintana* (28 and 64%, respectively [P = 0.003]). If culture is to be considered an efficient tool for the diagnosis of several *Bartonella*-related diseases, methodologies need to be improved, notably for the recovery of *B. henselae* from lymph nodes of patients with cat scratch disease.

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Human infections due to *Bartonella* species (formerly *Rochalimaea* species) are widely considered emerging diseases. They include long-recognized diseases such as Carrion's disease due to *Bartonella bacilliformis*, trench fever due to *Bartonella quintana*, and cat scratch disease (CSD) due to *Bartonella henselae* and *Bartonella clarridgeiae* (25, 32, 38). Newer clinical manifestations such as bacillary angiomatosis (BA) and peliosis hepatitis caused by both *B. henselae* and *B. quintana*; chronic lymphadenopathy due to *B. quintana*; and endocarditis due to *B. henselae*, *B. quintana*, and, in one case, *Bartonella elizabethae* have been recently identified (2, 38, 39). New molecular biology techniques, mainly based on 16S rRNA gene amplification and analysis, have allowed recognition of the role of *Bartonella* in an increasing number of pathological conditions. The association of *B. henselae* infection with BA is an example of such an approach for the identification of pathogens difficult to cultivate (52).

Growth of bartonellae is slow, since when blood agar is used (16, 34, 40, 60), primary isolates are typically obtained after 12 to 14 days, although prolonged incubation periods of up to 45 days are sometimes necessary (40). First subcultures of an isolate are also difficult to obtain, with colony formation again taking 10 to 15 days. Repeated subcultures, however, reduce this time to only 3 to 5 days, although colonial morphology is significantly affected. Cell coculture systems, as first described by Weiss et al. (64), have been reported to be more sensitive and allow more rapid growth of bartonellae than the blood agar-based techniques (16, 29, 43, 60).

Although the number of diagnosed cases of bartonella infections continues to increase, most diagnoses are based on either serology or PCR-based methods (1, 51), and reports of isolation of *Bartonella* from humans remain scarce. Furthermore, this finding is in contrast to the apparent ease with which *B. henselae* and *B. clarridgeiae* can be isolated from cats, their natural reservoir (8, 23, 28, 33). In this work, we summarize our 5-year experience (September 1993 to September 1998) of
attempted isolation of bartonellae from human clinical speci-
mens, during which time several techniques including cell cul-
ture and axenic media have been used.

MATERIALS AND METHODS

Specimen collection for Bartonella sp. diagnosis and case definition. As the National Reference Center for Rickettsioses, our laboratory receives approximately 10,000 samples each year from France and abroad with presumptive diagnoses of rickettsial disease, with 40% of them from our hospital center. We have developed several protocols for collection and management, each tailored to the patient’s sampling circumstances. When samples were obtained from hospit-
als other than those directly served by our laboratory, efforts were made to contact the sender, to arrange the collection of additional or more-suitable samples if necessary, and to obtain relevant clinical and epidemiological data. At least a serum sample was obtained from all patients. Most clinical data associated with the isolates described in this work have been described elsewhere (9, 12, 16, 17, 37, 40, 43, 44).

Patients were suspected of having Bartonella endocarditis if standard blood culture remained sterile. Diagnosis was based on fulfillment of the Duke’s endocar-
ditis service criteria (19) in which a positive serologic result was included as a minor criterion, and detection of bartonellae by culture and/or genotypic amplifi-
cation from blood and/or from valvular tissue was considered as a major crite-
ria. Complete sampling included a heparinized blood sample for shell vial and blood plating. Frozen cardiac valves were also sent when removed and inoculated onto shell vials and agar plates. When heparinized blood was not available, we asked that the inoculated blood culture bottle be submitted. Broth was further inoculated on shell vial and agar plates.

All homeless patients who presented for any reason to the emergency room of any hospital in Marseille, France, and who provided their informed consent were sampled. Samples included at least a heparinized blood sample for blood culture and a blood culture bottle for subculture on agar. Bartonella sp. bacteremia was de-
scribed below under “Agar plate procedure.” After publication of the study of
Rutherford, N.J.) was plated onto Columbia sheep blood agar plates as
preparations from stomach contents and subsequent from the biopsy samples were cultured in BHI broth (Becton Dickinson) and onto fresh Columbia sheep blood agar plates in order to establish isolates of Bartonella sp. A strain was considered established after two subcultures were obtained.

Identification of isolates. When bacteria were obtained on Columbia sheep blood agar plates, presumptive identification of isolates was made by determi-
nation of oxides and catalase reactions and by microscopic examination after
Gram and Gimez staining. When bacteria were obtained on shell vials, pre-
sumptive identification was made by immunofluorescence. DNA extracts were prepared from suspect colonies or from immunofluorescence-positive shell vials for use within Amplification with the QIAamp DNA Blood kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. During the first 3 years of this study, molecular detection and identification were based on 16S RNA gene and citrate synthase gene amplification and sequencing as previously described (26, 53). Subsequently, a new procedure for citrate synthase gene amplification was designed. This PCR incorporated the primers CS 443 (6) and CS 979 (TGC ATG ATT TTT GCA CTT G) designed by R. J. Birtles in our laboratory, allowing amplification of a citrate synthase (gltA) fragment. The identity of gltA gene products derived from test samples was determined by sequence determination and comparison as previously described (26, 53). Further confirmation of identity was achieved by intergenic spacer region amplification and sequence comparison. This PCR incorporated the primers QHVE1 (TGC TGA TGA TGC CAG GCC) and QHVE3 (AAC ATG TCT GAA TAT TTC) designed by V. Roux in our laboratory.

Molecular detection of Bartonella sp. from clinical samples. DNA extracts suitable for use as templates in PCRs were prepared from crushed tissues and frozen tissue and blood samples by using the reagents of the QIAamp DNA Blood kit (Qiagen) according to the manufacturer’s instructions. The effectiveness of the extraction and the absence of PCR inhibitors were assessed by PCRs incorpo-
rating human β-globin gene primers, as previously described (22). Positive con-
trol reaction mixtures for each assay incorporated DNA extracted from B. eliza-
betue. Negative control reaction mixtures were sterile distilled water. The procedures for DNA amplification and sequence determinations were the same as those used for the identification of isolates described above.

SeroLogic procedure. B. quintana Oklahoma, provided by D. F. Welch (Uni-
versity of Oklahoma, Norman), and B. henselae Houston (ATCC 49882) were pro-
gated in 150-cm² culture flasks containing ECV 304 human endothelial cell monolayers to be used as antigens for an immunofluorescence assay. All pro-
cedures for the B. henselae and B. quintana immunofluorescence assays have been described in detail elsewhere (16, 40).

Statistical tests. Pearson’s chi-square test was used to compare data. A dif-
ference was considered significant when P was <0.05. When arms were lower than 30, a difference was considered significant when P was <0.1.

RESULTS

Results are summarized in Tables 1 and 2. For each procedure studied, the sensitivity of the procedure was calculated by dividing the number of positives obtained by this procedure by the overall number of positives (number of positive detections by genomic amplification or any culture procedure in any sam-
ple).
subculture of broth in one, and by inoculation of heparinized blood in the second, demonstrating the complementarity of these procedures. No isolate was obtained by direct plating of blood onto agar. The sensitivities of each isolation protocol are presented in Table 2. The overall sensitivity of cell coculture procedures (28%) was significantly higher than that of agar procedures (5%) (\( P = 0.045 \)). With the exception of one patient who was treated for less than 24 h, none of the patients who had begun an antibiotic regimen, including rifampicin, fluoroquinolones, or aminoglycosides, had a positive blood culture. In the group of patients with PCR-positive detection on valvular tissue who received an antibiotic for more than 24 h prior to blood sampling, seven of seven had negative blood culture. Previous antibiotic therapy significantly affected results of blood culture, since none of the 32 patients with previous antibiotic therapy had a \( \text{Bartonella} \) strain established (one strain was isolated) from blood cultures, whereas four of five patients with no previous antibiotic therapy had their strain isolated and established from blood culture (\( P = 0.00006 \)).

Thirteen isolates from 12 patients were obtained from cardiac valves, 12 by using shell vial and 1 by using the agar procedure. All patients had positive serology and positive PCR results. All had received antibiotics for more than 2 days prior to sampling. None of these isolates could be successfully established. Among this group, the sensitivity of the agar procedure and that of the shell vial procedure were significantly different, being 4 and 44%, respectively (\( P < 0.00005 \)) (Table 3).

**Patients with suspected CSD.** A total of 80 heparinized blood samples, 250 lymph node biopsy samples, and 3 osteomedullary biopsy samples were obtained from 290 patients with suspected CSD. Among these patients, 78 were diagnosed as definitely having CSD. Only 5 patients yielded isolates, whereas the 73 remaining patients were diagnosed by serology or genomic detection (53 by serologic findings, 5 by genomic

### Table 1. Sensitivities of culture techniques used for the diagnosis of \( \text{Bartonella} \) infections

<table>
<thead>
<tr>
<th>Disease (( n ))</th>
<th>Result by:</th>
<th>No. of patients</th>
<th>No with:</th>
<th>B. henselae</th>
<th>B. quintana</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA (7)</td>
<td>+ NT(^a)</td>
<td>- 2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NT</td>
<td>+ 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - -</td>
<td>- 4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocarditis (38)</td>
<td>+ + +</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ NT</td>
<td>+ 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- NT</td>
<td>+ 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>+ 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- + +</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- + -</td>
<td>- 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSD (78)</td>
<td>+ + +</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ + -</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ - -</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- + +</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ - -</td>
<td>- 5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) NT, not tested.

### Table 2. Sensitivity of procedures used to isolate \( \text{Bartonella} \) sp. from blood of patients with \( \text{Bartonella} \) endocarditis

<table>
<thead>
<tr>
<th>Procedure (( n ))(^a)</th>
<th>No. positive</th>
<th>% Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparinized blood-shell vial (20)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Blood culture broth-shell vial (7)</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td>Total shell vial (25)</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Whole blood-agar plate (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood culture bottle-agar plate (6)</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Total agar (20)</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^{a}\) For each procedure used, \( n \) is the number of positive detections by genomic amplification or any culture procedure in any sample (overall positive). The significance (\( P \)) of total shell vial versus total agar is 0.045.

### Table 3. Sensitivity of the isolation procedures used in this study to isolate \( \text{Bartonella} \) sp. from valvular tissues of patients with \( \text{Bartonella} \) endocarditis

<table>
<thead>
<tr>
<th>Procedure (( n ))(^a)</th>
<th>No. positive</th>
<th>% Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell vial (27)</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>Agar (27)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Both (27)</td>
<td>12</td>
<td>44</td>
</tr>
</tbody>
</table>

\(^{a}\) For each procedure used, \( n \) is the number of positive detections by genomic amplification or any culture procedure in any sample (overall positive). The significance (\( P \)) of shell vial versus agar is <0.0005.
TABLE 4. Comparison of the sensitivities of the two methods used to isolate Bartonella sp. from the blood of bacteremic homeless patients

<table>
<thead>
<tr>
<th>Procedure (n)</th>
<th>No. positive</th>
<th>% Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood plating (49)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Blood culture broth plating (49)</td>
<td>48</td>
<td>98</td>
</tr>
<tr>
<td>Both (49)</td>
<td>49</td>
<td>100</td>
</tr>
</tbody>
</table>

* For each procedure used, n is the number of positive detections by any culture procedure (overall positive). The significance (P) of whole blood versus blood culture broth is <10⁻⁷.

Detection of B. henselae from lymph nodes, and 15 by both). B. henselae isolates were obtained from the lymph node biopsy samples of three patients with positive genomic detection for B. henselae. Only one had positive serology. One isolate was obtained by using both shell vial and agar, but this strain could not be subcultured. One isolate was obtained only in shell vial but could not be established. The third isolate was obtained on agar and was successfully passed. For the two patients with negative serology, the isolated strains were B. henselae serotype Marseille, and when this strain was used as antigen in immunofluorescence assay both had significant titers of antibody to this strain. Two B. quintana isolates were obtained from two patients with chronic lymphadenopathy and contact with cats and cat fleas. One strain was obtained from blood by using the shell vial (17). The second strain was isolated from an osteomediullar biopsy sample. PCR-based detection for B. quintana was positive in lymph node biopsy and blood samples (43). Both strains were successfully established. None of these patients had detectable antibodies against B. quintana. The sensitivities of agar plate and shell vial isolation procedures were estimated with 23 patients for whom sera was available, only one of seven had detectable antibodies against B. henselae. Of the BA patients for whom sera was available, only one of seven had detectable antibodies against B. quintana at the time that positive blood cultures were taken, but 10 seroconverted later in the study period.

DISCUSSION

The reliable diagnosis of Bartonella infections remains a difficult problem even for specialized laboratories. The isolation of infecting bacteria serves, therefore, not only as a means of diagnosis but also as a basis for the evaluation of much-needed improved diagnostic assays and as a route to enhanced understanding of the diversity and epidemiology of the Bartonellae and the infections that they cause. Genotypic and phenotypic differences between B. henselae strains have now been widely encountered (5, 15, 28, 37, 52), and antigenic variation may explain the inconsistent results in the serological diagnosis of diseases such as CSD (15, 18). The presence of at least two different genotypes of B. henselae in cats in Europe, whereas only one appears to be present in North America (5), resembles the situation for Borrelia burgdorferi sensu lato (63). The discovery that Borrelia garinii, Borrelia afzelii, and B. burgdorferi sensu stricto are prevalent in Europe, whereas only the latter is prevalent in North America, allowed the understanding that each species of the B. burgdorferi complex was responsible for specific clinical presentations. Furthermore, B. quintana and B. claridgeiae are responsible for some cases considered to be CSD (32, 38). The isolation of previously unrecognized pathogenic species, such as B. claridgeiae, further demonstrates the potential shortfalls of specific serological or PCR-based assays. Interestingly, it is also becoming clear that for “natural” vertebrate hosts of certain Bartonella species, be they humans (9, 17, 43) or small mammals, an immunological response to bacteremia appears to be absent. The clinical isolation rates of Bartonella species and in particular of B. henselae appear excessively low, especially if estimates of disease prevalence are accurate. Despite there being about 22,000 clinically diagnosed cases of CSD per year in the United States alone (24), and more and more rigorous and widespread attempts to obtain isolates during the past decade, only 13 B. henselae strains (including those of the present study) have been reported to be isolated from CSD patients (3, 13, 68). The present study includes 72 isolates of B. quintana and 8 isolates of B. henselae from 42 patients, of which 63 B. quintana and 2 B. henselae isolates were successfully established. None of these patients was infected by both species. The fact that B. henselae and B. quintana were the sole species isolated is probably due to the specificity of the antiserum that we used for the identification of the shell vials. Previously reported isolations by other laboratories included only B. henselae in 39 patients (47 isolates) and B. quintana in 22 patients (53 isolates) (10, 11, 13, 14, 20, 27, 29, 34–36, 42, 47–50, 55–60, 62, 65–68). However, Koehler et al. reported recently, in the largest series to date, the isolation of 36 Bartonella strains from 25 patients with BA, of which 9 were infected by B. henselae and 16 were infected by B. quintana (30).

The media and procedures which have been employed for the clinical isolation of Bartonella species have been highly variable and have given discrepant results. From published data, it appears that Bartonella spp. are well isolated on cho-
olate agar, especially when enriched with 20% sheep blood for *B. quintana* (13, 14, 27, 29, 34, 36, 47, 55–59, 62). *B. henselae* is also better isolated on CDC blood agar (13). Nevertheless, strains have also been isolated on heart infusion blood agar (14, 29, 34, 50), Trypticase soy blood agar (29, 50, 62), brucella blood agar (14), and Columbia blood agar (56) (present study). *B. quintana* has never been recovered with buffered charcoal yeast extract or brain heart infusion blood agar (10, 14, 34), whereas *B. henselae* has been isolated on brain heart infusion blood agar (10, 14, 50). The results of culture on buffered charcoal yeast extract agar give discrepant results (11, 14, 56).

Most strains have been isolated at 35 to 37°C in 3 to 9% CO2 atmosphere. On subculture, growth may be obtained at 30°C (55). Some authors report that no isolates are obtained at 30°C or without CO2 (56), whereas others report isolation and better growth at 30°C than at 35 to 37°C (11). Only one study has compared subculture media (10). The authors concluded that better growth was obtained on brain heart infusion blood agar and Columbia blood agar than on brucella blood agar, Shaefer's blood agar, and Trypticase soy agar (on which cultures were nevertheless obtained). Recently, Koehler et al. reported that optimal growth of *B. henselae* was obtained on heart infusion agar with 5% rabbit blood, whereas for *B. quintana* the best growth was obtained on chocolate agar (30). We tested two *B. quintana* strains, two *B. henselae* strains, the *B. elizabethae* strain, and a *Bartonella vinsonii* strain on various media including house-made Columbia agar, heart infusion agar, and brain heart infusion agar, all enriched with 5% horse blood, sheep blood, or rabbit blood, together with three commercial Columbia sheep blood agars. All plates were incubated at 35°C with 5% CO2 or under a microaerophilic atmosphere. All these media under both atmospheres supported the growth of all the strains tested, with no evident superiority of a specific medium (unpublished data). Nevertheless, for each medium the best growth was obtained when it was freshly prepared. In fact, these works are hampered by the fact that media are compared by subculture of well-established strains for which visible colonies are obtained after 3 or 4 days of incubation, whereas primary isolation is harder and may require up to 45 days. Although cocultivation of infected material with eukaryotic cell cultures has been shown to be an alternative to, and in some instances a more sensitive isolation method than, agar procedures (29, 30, 42), including for two *B. quintana* isolates of this study, the sole use of this approach cannot yet be advised as reports of agar growth success and concurrent cell culture failure continue, as reported in the present study for a *B. henselae* isolate.

We found that previous antibiotic therapy significantly adversely affected the outcome of blood culture attempts for patients with endocarditis, since all together, none of 32 patients with previous antibiotic therapy had a *Bartonella* strain established (one strain was isolated) from blood cultures, whereas 4 of 5 patients with no previous antibiotic therapy had their strain isolated and established from blood culture (*P* = 0.00006). Conversely, previous antibiotic therapy did not prevent isolations from being made from heart valve tissue but did prevent establishment of strains. In the 43 culture-positive patients of this study, among the 18 samples patients with previous antibiotic therapy, only 2 had their strain established, of whom 1 was treated for less than 24 h when sampled, whereas among the 25 patients sampled with no previous antibiotic therapy, a sole of *B. henselae* strain isolated from lymph node patients with CSD was lost on subculture. Difficulties in the successful subculture of *Bartonella* isolates have also been encountered elsewhere (62). This finding may be more frequent than is currently recognized; several groups whom we contacted in order to obtain reported strains told us of losses on subculture.

For patients with endocarditis, inoculation of blood culture broth and heparinized blood onto shell vial was significantly more effective for the recovery of isolates than were the agar procedures employed. Furthermore, as inoculation of blood culture broth and inoculation of heparinized blood onto shell vial appeared complementary, they should both be attempted. Isolation of *B. quintana* from the blood of homeless patients appears to be straightforward, with up to 11 isolates for a single patient during a 1-month period. This apparent case, compared to the situation for patients with endocarditis, may result from the lack of previous antibiotic therapy in the former group. For the diagnosis of bacteremia in homeless individuals, the use of an automated blood culture system followed by systematic blood culture broth was demonstrated to be highly effective and was significantly more sensitive than direct blood plating. Nevertheless, these procedures should be used together, since one bacteremic homeless individual yielded isolates only on direct plating. In previous studies, the lysis-centrifugation procedure and blood culture bottles with further subculture have been used for isolation of *B. quintana* and *B. henselae* from blood (10, 14, 20, 29, 34, 35, 47, 49, 50, 55, 56, 59, 62, 68). Both procedures were used together in two studies but gave discrepant results. In one study, isolation was assessed by the lysis-centrifugation procedure only (55), whereas in the other, higher isolation rates were obtained by using blood culture bottles with further subculture (34). However, as detection of these slowly growing bacteria on conventional broth blood culture is difficult because they produce little or no CO2 or visible growth, it is important to systematically subculture broth on solid medium for at least 1 month. In one study, the authors report that the optimum isolation rate was obtained by a combination of inoculation of blood culture bottle and lysis-centrifugation of blood culture broth (three isolates) rather than direct subculture of blood culture bottle (one isolate) (62). In this study, two isolates were detected in blood culture bottles but were not established. We observed the same problem for two endocarditis cases when subcultures on agar plates remained negative whereas subcultures onto ECV 304 monolayers yielded two *B. quintana* strains. The isolation rate of *B. quintana* from bacteremic homeless individuals was found to be higher when blood was frozen prior to inoculation, a methodology previously reported for blood samples from cats (8), which may be related to the intracellular location of bacteria in blood (31).

Shell vials were significantly more effective than agar medium for the recovery of *Bartonella* sp. from cardiac valves. For BA patients, the recovery of *B. quintana* from biopsy material was efficient, whereas the recovery of *B. henselae* was not. Difficulty in isolating *B. henselae* from BA patients was also observed in the study by Koehler et al. (30). By reviewing all cases of BA in which the infecting *Bartonella* organism was identified to species level (4, 7, 20, 21, 30, 41, 52, 54, 55, 57), we found that *B. quintana* was cultured from 21 of 33 cases (sensitivity, 64%), whereas *B. henselae* was cultured from only 10 of 36 cases (sensitivity, 28%) (*P* = 0.003). The recovery of *B. henselae* isolates from the lymph nodes of patients with CSD is extremely difficult. In our study, of 78 CSD patients, 69 had elevated antibody titers against *B. henselae* and 22 were positive by PCR amplification of *B. henselae* from DNA extract prepared from lymph nodes, but *B. henselae* was isolated in only three cases, two isolates belonging to the Marseille serogroup (15, 37). Furthermore, only one of these isolates could be established. Similar results were obtained by Avidor et al., who isolated two *B. henselae* strains from 29 PCR-positive
TABLE 5. Comparison of the sensitivities of culture for the diagnosis of B. henselae infection in three clinical syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>No. genomic detection</th>
<th>No. genomic detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive and isolation</td>
<td>positive and isolation</td>
</tr>
<tr>
<td>BA</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CSD</td>
<td>3</td>
<td>20</td>
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</tbody>
</table>

* Data regarding CSD and endocarditis patients were obtained during this study, whereas data regarding BA patients have been drawn from both this study and previously reported cases (4, 7, 20, 21, 30, 41, 52, 54, 55, 57). Culture sensitivity for BA and endocarditis groups combined was 33%; culture sensitivity for the CSD group was 13%. The values for the BA and endocarditis groups were significantly different from those for the CSD group (P = 0.084).

lymph nodes of patients with CSD (3). These observations cannot solely be due to the fastidious nature of B. henselae, which is harder to isolate than B. quintana, as B. henselae is significantly harder to isolate from lymph nodes of CSD patients than from the blood of patients with B. henselae endocarditis or from skin biopsy samples of patients with BA (Table 5). The presence of pus in affected lymph nodes could explain the lack of viability of B. henselae. The difficulty in isolating B. henselae from lymph nodes is also starkly contrasted with the apparent ease of its isolation from cat blood (8, 28, 31, 33). This finding closely resembles that observed for homeless patients with B. quintana in our study and another study (60), for whom isolation of B. quintana appeared to be simple. This paradox raises several questions including (i) the viability of the bacterium when out of circulating blood, (ii) the presence in blood of a soluble and labile factor essential for growth of some isolates, and (iii) the heterogeneity of culture requirements of Bartonella strains.

An alternative procedure for diagnosing Bartonella infections is the use of PCR-based detection methods. These had a sensitivity of 81% on cardiac valves, and diagnosis was made for seven patients for whom all cultures remained negative. However, among CSD patients, the sensitivity of PCR methods in detecting DNA of B. henselae was lower (30%), including five cases in which both serology and culture were negative. The sensitivity of genomic detection of Bartonella from tissue biopsy samples of BA patients was 100% and allowed the identification of the responsible species, facilitating epidemiological investigations (30). Genomic detection was not performed with blood of bacteremic homeless patients due to the low sensitivity of this procedure on blood in our experience, but a good procedure to detect such patients should be the genomic detection of B. quintana from body lice as previously demonstrated (9, 45, 46).

The most effective tool for the diagnosis of endocarditis and CSD is specific serology. For patients with endocarditis, the serology had a sensitivity of 97%. Furthermore, 95% of these patients had an antibody titer against Bartonella sp. of ≥1:1,600. The sensitivity was 90% for patients with B. henselae CSD. We found, however, that serology has no value in the diagnosis of BA, as only one of seven was positive. This result is probably due to the immunocompromised status of these patients (30). Furthermore, although seroconversion was observed for 10 of the homeless patients with bacteremia, all had no detectable antibodies at the time at which samples which yielded cultures were taken. Serology is therefore reaffirmed in this case as a tool for retrospective rather than for acute-phase diagnosis.

We can now conclude from all these data that there is to date no universal optimal procedure for the isolation of B. quintana and B. henselae, but rather several techniques should be combined to achieve the observed disease. In the future, only prospective studies using several media and procedures will allow a definition of optimal conditions for isolating Bartonella species, and the most pressing problem is perhaps improved methods for the recovery of isolates from CSD patients.

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