Association of Bartonella spp bacteremia with Chagas cardiomyopathy, endocarditis and arrythmias in patients from South America

Association of *Bartonella* spp bacteremia with Chagas cardiomyopathy, endocarditis and arrhythmias in patients from South America

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Abstract

Infection with *Bartonella* spp may cause cardiac arrhythmias, myocarditis and endocarditis in humans. The aim of the present study was to evaluate a possible association between *Bartonella* spp bacteremia and endocarditis, arrhythmia and Chagas cardiomyopathy in patients from Brazil and Argentina. We screened for the presence of bacterial 16S rRNA in human blood by PCR using oligonucleotides to amplify a 185-bp bacterial DNA fragment. Blood samples were taken from four groups of subjects in Brazil and Argentina: i) control patients without clinical disease, ii) patients with negative blood-culture endocarditis, iii) patients with arrhythmias, and iv) patients with chronic Chagas cardiomyopathy. PCR products were analyzed on 1.5% agarose gel to visualize the 185-bp fragment and then sequenced to confirm the identity of DNA. Sixty of 148 patients (40.5%) with cardiac disease and 1 of 56 subjects (1.8%) from the control group presented positive PCR amplification for *Bartonella* spp, suggesting a positive association of the bacteria with these diseases. Separate analysis of the four groups showed that the risk of a Brazilian patient with endocarditis being infected with *Bartonella* was 22 times higher than in the controls. In arrhythmic patients, the prevalence of infection was 45 times higher when compared to the same controls and 40 times higher for patients with Chagas cardiomyopathy. To the best of our knowledge this is the first report of the association between *Bartonella* spp bacteremia and Chagas disease. The present data may be useful for epidemiological and prevention studies in Brazil and Argentina.

Key words: Human; PCR; *Bartonella* spp; Chagas disease; Endocarditis; Arrhythmia

Introduction

The Gram-negative organisms of the *Bartonella* genus are fastidious micro-aerobic bacteria classified into the second subgroup of the Proteobacteria class (1). This genus contains about 30 species, some of which have been isolated from humans (*B. bacilliformis*, *B. henselae*, *B. elizabethae*, and *B. quintana*) and from cats and dogs (*B. koehlerae* and *B. vinsonii berkhoffii*). Most known species of *Bartonella* have been isolated from rodent blood (2,3).

The life cycle of *Bartonella* spp consists of a reservoir host and vectors (e.g., ticks, fleas and lice) that transmit bacteria from the reservoir to novel hosts (3). Transmission may also occur through shared needles and syringes used by drug users. Hosts accidentally infected with *Bartonella* spp can show systemic signs, such as bacteremia and cardiac diseases mostly related to abnormalities in the heart valves, which may result in endocarditis (4,5). Surgery may be required in 20-40% of patients with infective endocarditis, mostly for valve replacement (5,6).

Ticks are considered to be zoonotic vectors that cause several diseases, and the tick *Ixodes pacificus* in particular...
may play an important role in the transmission of *Bartonella* to both humans and animals (2). *Bartonella* has been found in the blood of residential animals (domestic cats and dogs) and rodents, which are regarded as vectors and transmitters of several diseases (7,8). *B. v. berkholffi* was isolated from a domestic dog with endocarditis (9), and an epidemiological study demonstrated that *B. v. berkholffi* infection frequently occurs through ticks (10). *B. v. berkholffi* and *B. henselae* were isolated from cerebrospinal fluid and periodontal swab samples from a patient who had contact with infected animals (11).

*B. bacilliformis* has also been found in erythrocytes, resulting in a severe hemolytic anemia known as Oroya fever (12). The cat-scratch disease, which is mainly caused by *B. henselae*, has been described as the most common *Bartonella* infection in humans worldwide (13). *B. quintana* infection was described during World War I, leading to trench or quintana fever (13), and there have been recent reports of urban trench fever (14,15). The symptoms of *Bartonella* infection include fever, fatigue, insomnia, joint pain, headache, weight loss, cardiac arrhythmias, and muscle pain (16). The major risk factors for *B. quintana* include infections, precarious conditions and chronic alcoholism. These risk factors are the same as those found for human immunodeficiency virus (HIV) and endocarditis (17). Therefore, although endocarditis caused by *B. quintana* has been described as the most common *Bartonella* infection in humans worldwide (13), *B. quintana* infection was described during World War I, leading to trench or quintana fever (13), and there have been recent reports of urban trench fever (14,15). The symptoms of *Bartonella* infection include fever, fatigue, insomnia, joint pain, headache, weight loss, cardiac arrhythmias, and muscle pain (16). The major risk factors for *B. quintana* include infections, precarious conditions and chronic alcoholism. These risk factors are the same as those found for human immunodeficiency virus (HIV) and endocarditis (17). Therefore, although endocarditis caused by *B. quintana* has been underestimated, the presence of *Bartonella* infection should be further investigated in patients with negative blood culture and epidemiological antecedents related to chronic alcoholism, precarious conditions, contact with cats, fleas and lice, and in patients with endocarditis and positive serology for *Chlamydia* sp (18). *B. quintana*, *B. henselae* and *B. elizabethae* were first described as the causal agents of endocarditis in humans. Two other subspecies of *B. vinsoni*, *berkholffi* and *arupensis*, were later found to be associated with endocarditis in patients with existing valvulopathies (14,18). *B. henselae*, which leads to endocarditis in children, exhibits negative results in serological tests (19). A number of studies in European countries have recently demonstrated that *Coxiella burnetii*, *B. henselae* and *B. quintana* are significantly associated with endocarditis in patients with negative blood cultures (20). Siciliano et al. (21) showed that, of 61 assessed patients with endocarditis, 17 (27%) still had negative blood cultures. We reason that there are many negative blood cultures because the bacteria grow slowly and only in enriched medium (usually colonies are seen only after 20 days), which are conditions not routinely used in diagnostic laboratories. Serological tests are still helpful for diagnosis, but serum cross-reactivity between *Bartonella*, *Chlamydia*, and *Coxiella* species may interfere with the diagnosis and choice of treatment (6,20). Thus, molecular techniques such as PCR may be critical in providing a faster and more specific diagnosis of the disease (15,22). More recently, a multiplex PCR assay was developed to detect more than 20 different known and some unknown *Bartonella* species in both clinical and environmental samples (23). The gene targets for PCR amplification for the identification of specific species of *Bartonella* are either the conserved 16S rRNA, a generic marker for detecting any *Bartonella* species, or the intergenic transcribed spacer 16S-23S rRNA (23).

The medical significance of *Bartonella* species as human pathogens has been demonstrated by epidemiological, natural history, pathological, and host-microbial interaction studies (11,24). Patient co-infection by *B. henselae* and *Borrelia burgdorferi* has also been described (25) and therefore the association of *Bartonella* spp with other zoonoses must be investigated. Chagas disease is an important zoonosis in South America that frequently evolves to cardiomyopathy (26). To gain a better understanding of the consequences of *Bartonella* infection, we have analyzed samples from Brazilian and Argentinian patients with a single-step PCR assay. The subjects of this study, classified as heart disease patients, presented negative blood-culture endocarditis, relevant arrhythmias or Chagas cardiomyopathy. Our results suggest a strong association between the presence of *Bartonella* spp and these diseases. To the best of our knowledge this is the first report of such data from South America.

**Material and Methods**

**Ethical aspects**

The current project was approved by the Ethics Committee of Universidade Federal de São Carlos (UFSCar, CAAE #0024.1.135.000-06) and all subjects gave written informed consent to participate.

**Patients and controls**

This study investigated 56 normal voluntary subjects (control group) without cardiac disease and 148 voluntary subjects with heart diseases (46 cases with negative blood-culture endocarditis, 29 with relevant cardiac arrhythmias and 73 with chronic Chagas cardiomyopathy) between 2004-2008. These subjects were assessed and selected by cardiologists from Brazil and Argentina. Each subject was asked to complete a standard questionnaire, which included age, gender, previous contact with animals and arthropods, clinical symptoms, and eventual antibiotic treatment. The control group included 32 volunteers from a medical course in the city of Paracatu, Minas Gerais State, and 24 from the city of São Carlos, in São Paulo State, Brazil. Among this last group, 10 were from a rest home for the elderly and did not present any detectable heart problems. The patients with arrhythmia and endocarditis were from the cities of São Carlos (SP, 10 cases) and from the city of São Paulo (SP, 65 cases). Fifty-two cases of Chagas cardiomyopathy were selected from the University Hospital, Ribeirão Preto (SP), Brazil. Twenty-one patients with chronic Chagas cardiomyopathy from Argentina were selected from the locality of Aréaty, Santiago del Estero, a highly endemic region in Argentina. Thirty-two asymptomatic subjects but seropositive for Chagas disease were selected from blood donors admitted to Hemoenaran Buenos Aires and to the service of Obstetrics of Rivadavia Hospital. All of them were residents of the metropolitan area of Buenos Aires but 6 were originally from Bolivia.
3 from Paraguay, and 6 from the Chaco region (26).

**Blood sample collection from the subjects**

A 10-mL sample of venous blood was collected into vacuum tubes containing 72 USP units of EDTA/tube that was used for extraction of genomic DNA. DNA was extracted from 500 µL total blood according to modifications of the methodology described by Lahiri and Nurnberg Jr. (27). Alternatively, 10 mL peripheral blood was collected from Chagas disease patients and immediately mixed with one volume of 2X lysis buffer containing 6 M guanidinium hydrochloride and 0.2 M EDTA, pH 8.0 (GE) (28). The GE-blood lystate (GEB) was boiled (29) and stored at 4°C. DNA was purified from 100 µL aliquots of GEB by phenol extraction and isopropanol precipitation, as previously reported (30).

**DNA amplification**

Genomic DNA from blood was used as template for PCR to confirm the presence of *Bartonella* spp. The primers used in the PCR were designed as previously described (7). The primer sequences were Bh16SF (5′-AGAGTTGTATCTGGCTCAG-3′) and Bh16SR (5′-CCGATAATCCTTTCCCTCCTAA-3′), which amplify a 185-bp fragment of the 16S rRNA gene of *B. henselae* strain Houston-1 (GenBank accession No. NC_005956). The primers P1-5EZ (5′-ATAATCACATGGAGAGCCACAAGCT-3′) and P2-3EZ (5′-GCACCTTTTTGGTATCTGAGAAAGT-3′), which amplify a 447-bp product of the ZFY and ZFX human genes, were used to assess the DNA integrity of the extracted blood samples (31). Each reaction contained 1 µg DNA, 0.2 µg of each primer, 250 µM of each dNTP, 10 mM Tris-HCl, pH 8.5, 25 mM KCl, 5 mM (NH₄)₂SO₄, 2 mM MgCl₂, and 1.5 U Taq DNA polymerase in a final volume of 100 µL.

The PCR conditions were as follows: 35 cycles of 95°C for 30 s, 54°C for 1 min, and 72°C for 45 s, followed by a final extension of 10 min at 72°C. The amplification products were analyzed on 1.5% agarose gels in the presence of ethidium bromide. A negative control was included in each PCR run to avoid false results. Bands from representative samples corresponding to the 185-bp fragment were removed from the agarose gels, purified using the Perfectprep Gel Cleanup Kit (Eppendorf, Germany) and sequenced. The samples were prepared using the DYEneramic™ ET Dye Terminator Kit (GE Healthcare®, Sweden) with the same primers. The reactions were cycled (95°C for 20 s, 50°C for 15 s, and 60°C for 1 min), precipitated using ammonium acetate and ethanol, washed with 70% ethanol, eluted in 10 µL loading solution, and run on a MegaBACE™ DNA Analysis System (GE Healthcare®).

**DNA data analysis**

The sequences obtained were analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) (32) and compared to each other with the MULTALIN program (http://prodes.toulouse.inra.fr/multalin/multalin.html) (33).

**Statistical analysis**

The correlation between DNA amplification for *Bartonella* spp and human heart diseases was analyzed by the chi-square test. This test was used to determine if the observed frequency of heart disease associated with *Bartonella* bacteremia was significantly different from the distribution of expected heart disease frequency. In addition, the odds ratio was also calculated to measure the strength of association or non-independence between the two calculated frequencies (34).

**Results**

**Study patients and controls**

A total of 204 subjects were selected clinically and DNA samples were obtained from their blood (Table 1). The most important criteria for inclusion in the heart disease group were the presence of blood-negative endocarditis, relevant arrhythmias or Chagas cardiomyopathy. Patients with endocarditis or arrhythmias were serum negative for Chagas disease. Most subjects reported to have had previous contact with animals.

| Table 1. Distribution of the 204 subjects who participated in this study from April 2004 to June 2008. |
|---|---|---|---|---|---|
| Groups | N/group | City/State | Age | Gender | Contact with animals |
| **Brazil** | | | | | |
| Control group | 32 | Paracatu, MG | 22.3 ± 4.0 | 15 M, 17 F | ~50% |
| | 24 | São Carlos, SP | 47.2 ± 21.4 | 8 M, 16 F | 100% |
| **Case groups** | | | | | |
| Endocarditis | 46 | São Paulo, SP | 53.5 ± 15.1 | 26 M, 20 F | ~60% |
| Arrhythmias | 19 | São Paulo, SP | NI | 12 M, 7 F | 100% |
| Arrhythmias | 10 | São Carlos, SP | 61.8 ± 23.7 | 3 M, 7 F | 100% |
| Chagas cardiomyopathy | 52 | Ribeirão Preto, SP | 71.3 ± 12.9 | 36 M, 16 F | NI |
| **Argentina** | | | | | |
| Chagas cardiomyopathy | 21 | Buenos Aires | 37.9 ± 6.9 | 9 M, 12 F | NI |
| Chagas asymptomatic | 32 | Buenos Aires | 41.8 ± 8.7 | 16 M, 16 F | NI |

Data are reported as means ± SD. Place of blood donation. Four members of the endocarditis group were treated with nonspecific antibiotics before blood collection. Asymptomatic Argentinian subjects (N = 32) were used only for comparison with Chagas disease patients and not included in the total. NI = not informed.
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(dogs, cats, and horses) but this was not an exclusive criterion since it was highly variable along their lifetime. The most frequent diagnoses in the endocarditis group were mitral insufficiency, aortic stenosis, mitral stenosis, and mitral dysfunction, among others. Four cases of endocarditis were treated with nonspecific antibiotics before blood collection. Data from a group of 32 asymptomatic Argentinean subjects but with positive serology for Chagas disease were also obtained and used only for comparison with Chagas disease patients. However, this group was not considered to be either a control or case group and therefore it was not included in the total.

Amplification of human DNA samples

Patient DNA was subjected to PCR with the described oligonucleotides and the products were analyzed on a 1.5% agarose gel for the detection of 185- and 447-bp fragments. All reactions showed the positive control fragment of 447 bp. The 185-bp fragment, corresponding to the 16S rRNA gene of Bartonella, was present in 1 (1.8%) subject from the control group and in 60 (40.5%) subjects from the group of patients (Table 2; Figure 1A and B). The odds ratio and calculated chi-square are shown in Table 3. The odds ratio test showed a strong association between all groups of patients and the presence of bacteria. The risk of the Brazilian and Argentinean patients being infected with Bartonella spp was 37 times higher compared to control.

Data were also analyzed by Brazilian subgroups of the diseases. In this case, the risk of Bartonella spp bacteremia was 21.7 times higher for patients with endocarditis, 47.1 times higher for patients with arrhythmias, and 40.3 times higher for Chagas cardiomyopathy patients (Table 3). The risk of an Argentinean patient with Chagas cardiomyopathy to be infected with Bartonella was not significantly different from that of a Brazilian patient with the same disease. No statistical difference was found between Argentinean asymptomatic subjects with positive serology and the group of Argentinean patients with Chagas cardiomyopathy.

DNA sequencing of PCR products

Representative sequences (N = 35) from each group obtained from the PCR products were compared to the sequences deposited in public databases and confirmed the presence of Bartonella spp in the subjects. Figure 2 shows an alignment of representative sequences from 10 different positive subjects. Only two substitutions were found for this group in the sequence spanning position +13 to +193 bp. The consensus sequence of this alignment was compared to bacterial sequences at GenBank with the highest hits (e-value of 9e-06) with the 16S ribosomal RNA from several Bartonella species (Table 4). The majority of sequenced samples showed greater similarity to B. henselae, but bacteremic subjects could be found with B. vinsonii, B. v. berkhoﬀi and B. quintana sequence similarity.

Table 2. Amplification of 16S rRNA for the detection of Bartonella spp in healthy volunteers (controls) and patients with heart diseases from Brazil and Argentina.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of samples*</th>
<th>Positive PCR (16S rRNA)</th>
<th>Negative PCR (16S rRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>56</td>
<td>1 (1.8%)</td>
<td>55 (98.2%)</td>
</tr>
<tr>
<td>Cases#</td>
<td>148</td>
<td>60 (40.5%)</td>
<td>88 (59.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>61 (29.9%)</td>
<td>143 (70.1%)</td>
</tr>
</tbody>
</table>

*Samples from the two countries. #Asymptomatic subjects from Argentina were not included.

Figure 1. Analysis of the products obtained by PCR amplification of a fragment of the Bartonella spp 16S rRNA gene from DNA extracted from whole blood of humans with endocarditis (A) and subjects from the control group (B). Lane 1 = Ladder 1-kb marker; lanes 2 to 6 = PCR products in representative samples from 5 individuals. The 185- and 447-bp fragments (lower and upper bands) refer to the amplification of bacterial 16S rRNA and control gene, respectively.

Discussion

The incidence of negative blood-culture endocarditis cases ranges from 3 to 31% of all cases of endocarditis (20,35). Zoonoses and bacterial infections transmitted by arthropods are now recognized as emerging infectious agents that could cause endocarditis. In the present study, we analyzed 46 culture-negative endocarditis patients and...
found that 13 (28%) were positive for \textit{Bartonella} spp. These results are consistent with previous data, which suggest that \textit{Bartonella} infection is observed in 28 to 50% of all culture-negative endocarditis cases (20,35). \textit{Bartonella} infection is probably underdiagnosed due to nonspecific symptoms such as fever, weight loss and muscle pain. However, symptoms may become progressively more severe with eventual neurological symptoms if patients are not treated (11). Embolic phenomena and glomerulonephritis have also been described as complications of \textit{Bartonella} infection (18,20). Serology has been shown to be useful in confirming the presence of \textit{C. burnetii} and \textit{Bartonella} species, but the PCR technique proved to be more sensitive and specific for the diagnosis of specimens such as \textit{Tropheryma whipplei}, \textit{Bartonella} and fungi in blood and valvular biopsies (36).

There are few reports on the association of \textit{Bartonella} and cardiac malignant arrhythmias in dogs (7) or humans (8). It was suggested that \textit{Bartonella} spp induced silent subacute myocarditis and electric instability, which could be the major

\begin{table}
\centering
\caption{Association of heart disease and the presence of bacteria in the blood of patients.}
\begin{tabular}{lllllll}
\hline
Groups & No. of subjects & Positive PCR & Negative PCR & OR & 95\%CI & P  \\
\hline
Without heart disease, total & 56 & 1 & 55 &  &  &  \\
Heart disease, total & 148 & 60 & 88 & 37.50 & 5.4-748.7 & <0.001*  \\
Chagas disease, total & 73 & 34 & 39 & 47.95 & 6.5-981.0 & <0.001*  \\
Brazilian subjects &  &  &  &  &  &  \\
Controls & 56 & 1 & 55 &  &  &  \\
Endocarditis & 46 & 13 & 33 & 21.67 & 2.7-463.8 & <0.001*  \\
Arrhythmias & 29 & 13 & 16 & 44.69 & 5.3-986.2 & <0.001*  \\
Chagas cardiomyopathy & 52 & 22 & 30 & 40.33 & 5.3-642.8 & <0.001*  \\
Argentinean subjects &  &  &  &  &  &  \\
Chagas cardiomyopathy & 21 & 12 & 9 &  &  &  \\
Chagas asymptomatic & 32 & 11 & 21 & 2.55 & 0.71-9.28 & 0.17##  \\
Chagas cardiomyopathy &  &  &  &  &  &  \\
Brazilian patients & 52 & 22 & 30 &  &  &  \\
Argentinean patients & 21 & 12 & 9 & 0.55 & 0.17-1.72 & 0.37###  \\
\hline
\end{tabular}
\end{table}

OR = odds ratio; CI = confidence interval. *Compared to control. ##Compared to Argentinean subjects with Chagas cardiomyopathy; ###compared to Brazilian subjects with Chagas cardiomyopathy.
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Pathogenic factor in the development of arrhythmogenic right ventricular cardiomyopathy-like disease. However, the association between Bartonella spp infection and the onset of arrhythmias is not yet well understood. In this study, we show that 13 of 29 patients (45%) with arrhythmias were also positive for Bartonella, suggesting a strong association between bacterial infection and the disease.

Chagas disease (American trypanosomiasis) is still a major health concern in South America. According to the World Health Organization, 10 million people are infected with Trypanosoma cruzi worldwide, mostly in Latin America (37). Up to 30% of chronically infected people develop cardiac disorders and up to 10% develop digestive, neurological, or mixed alterations, for which specific treatment may become necessary. Here we demonstrate a strong association between Chagas cardiomyopathy and Bartonella infection in Brazilian and Argentinean patients. Also, there was no statistically significant difference between asymptomatic Argentinean subjects with positive serology and the group of Argentinean patients with Chagas cardiomyopathy. These results suggest a role for the vector of American trypanosomiasis, mostly Triatoma infestans, in transmitting Bartonella or other bacteria. This possibility should be investigated in order to achieve a better understanding of the association of Chagas disease and Bartonella bacteremia. The high prevalence of positive subjects in the Chagas disease group strongly suggests that a method for controlling this zoonosis is necessary. The contribution of Bartonella spp infection to the evolution of Chagas disease or to the intensity of symptoms remains to be determined.

One in 56 asymptomatic subjects that donated blood for this study had Bartonella spp bacteremia. Asymptomatic bacteremia had been already reported in blood donors (38). Immunodeficient patients, as HIV seropositive patients or patients receiving corticotherapy, can have angioproliferative reactions as seen in bacillary angiomatosis or bacillary peliosis hepatic as well fatal Bartonella spp infection (39). Further studies will be necessary to establish the relevance of Bartonella spp transmission by transfusion, especially in immunodeficient patients.

Our data show that Bartonella spp may be important etiologic agents of culture-negative endocarditis and should be investigated in all culture-negative endocarditis cases as well as in other suspected conditions. Our results also suggest a strong association between Bartonella spp bacteremia and arrhythmia or Chagas cardiomyopathy. As far as we know, this is the first report of such data from South America.

Acknowledgments

The authors are grateful to Dr. João Santana da Silva, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil, for providing the samples from patients with Chagas disease. Research supported by FAPESP and CNPq.

Table 4. Best BLAST results for the PCR product sequences of representative samples of each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Retrieved BLAST number</th>
<th>Number of individuals with identical sequences within the same group</th>
<th>Species with the highest hit</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 10)</td>
<td>DQ228135.1</td>
<td>4</td>
<td>B. vinsonii berkhofii</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>DQ645425.1</td>
<td>3</td>
<td>B. henselae</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>U26258.1</td>
<td>2</td>
<td>B. vinsonii</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>M11927</td>
<td>1</td>
<td>B. quintana</td>
<td>97</td>
</tr>
<tr>
<td>Endocarditis (N = 9)</td>
<td>DQ641913.1</td>
<td>4</td>
<td>B. henselae</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>AF143446.1</td>
<td>3</td>
<td>B. vinsonii berkhofii</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>U26258.1</td>
<td>2</td>
<td>B. vinsonii</td>
<td>98</td>
</tr>
<tr>
<td>Arrhythmia (N = 8)</td>
<td>DQ645425.1</td>
<td>4</td>
<td>B. henselae</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>DQ641913.1</td>
<td>3</td>
<td>B. vinsonii berkhofii</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>U26258.1</td>
<td>2</td>
<td>B. vinsonii</td>
<td>98</td>
</tr>
<tr>
<td>Chagas disease (N = 8)</td>
<td>DQ645425.1</td>
<td>4</td>
<td>B. henselae</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>DQ228135.1</td>
<td>2</td>
<td>B. vinsonii berkhofii</td>
<td>99</td>
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<tr>
<td></td>
<td>U26258.1</td>
<td>2</td>
<td>B. vinsonii</td>
<td>99</td>
</tr>
</tbody>
</table>

N = number of representative PCR products that were sequenced for each group. B. = Bartonella.
References

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