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# DETECTION OF *Bartonella henselae* DNA IN CLINICAL SAMPLES INCLUDING PERIPHERAL BLOOD OF IMMUNE COMPETENT AND IMMUNE COMPROMISED PATIENTS BY THREE NESTED AMPLIFICATIONS

**Karina Hatamoto KAWASATO(1), Léa Campos de OLIVEIRA(2), Paulo Eduardo Neves Ferreira VELHO(3), Lidia YAMAMOTO(4), Gilda Maria Barbaro DEL NEGRO(5) & Thelma Suely OKAY(4)**

## SUMMARY

Bacteria of the genus *Bartonella* are emerging pathogens detected in lymph node biopsies and aspirates probably caused by increased concentration of bacteria. Twenty-three samples of 18 patients with clinical, laboratory and/or epidemiological data suggesting bartonellosis were subjected to three nested amplifications targeting a fragment of the 60-kDa heat shock protein (HSP), the internal transcribed spacer 16S-23S rRNA (ITS) and the cell division (FtsZ) of *Bartonella henselae,* in order to improve detection in clinical samples. In the first amplification 01, 04 and 05 samples, were positive by HSP (4.3%), FtsZ (17.4%) and ITS (21.7%), respectively. After the second round six positive samples were identified by nested-HSP (26%), eight by nested-ITS (34.8%) and 18 by nested-FtsZ (78.2%), corresponding to 10 peripheral blood samples, five lymph node biopsies, two skin biopsies and one lymph node aspirate. The nested-FtsZ was more sensitive than nested-HSP and nested-ITS ($p < 0.0001$), enabling the detection of *Bartonella henselae* DNA in 15 of 18 patients (83.3%). In this study, three nested-PCR that should be specific for *Bartonella henselae* amplification were developed, but only the nested-FtsZ did not amplify DNA from *Bartonella quintana*. We conclude that nested amplifications increased detection of *B. henselae* DNA, and that the nested-FtsZ was the most sensitive and the only specific to *B. henselae* in different biological samples. As all samples detected by nested-HSP and nested-ITS, were also by nested-FtsZ, we infer that in our series infections were caused by *Bartonella henselae*. The high number of positive blood samples draws attention to the use of this biological material in the investigation of bartonellosis, regardless of the immune status of patients. This fact is important in the case of critically ill patients and young children to avoid more invasive procedures such as lymph nodes biopsies and aspirates.

**KEYWORDS:** Bartonellosis; *Bartonella* spp.; *Bartonella henselae*; Cat Scratch Disease, PCR.

## INTRODUCTION

Bacteria of the genus *Bartonella* are considered as emerging pathogens of increasing importance and worldwide distribution. The genus *Bartonella* includes at least 30 species and subspecies, and around 12 are human pathogens (BOULOUIS *et al.,* 2005; LAMAS *et al.,* 2008) responsible for syndromes such as Carrion's disease caused by *Bartonella bacilliformis* (nonexistent in non Andean countries), trench fever by *Bartonella quintana,* cat-scratch disease (CSD), bacillary angiomatosis, along with recurrent bacteremia, septicemia, endocarditis, trombocytopenic purpura, erythema multiforme, erythema nodosum, severe anemia and peliosis hepatis (BREITSCHWERDT *et al.,* 2010). *Bartonella henselae* is the most frequently identified species in human infections (LAMAS *et al.,* 2008; BREITSHWERDT *et al.,* 2010).

Laboratory diagnosis is based on culture isolation, serology, histopathology, and molecular detection mainly in lymph node biopsies and aspirates, (which is probably due to a higher concentration of bacteria in these specimens), and more rarely in peripheral blood samples (HANSMANN *et al.,* 2005; DEL PRETE *et al.,* 2000; ARVAND & SCHÄD, 2006). Species identification and differentiation is troublesome due to the bacteria's slow and fastidious growth. At the time of blood collection, the level of contamination is between 1 to 10 colony forming units/mL thus justifying the failure to isolate or amplify the bacterium DNA in blood samples after one-round amplifications (BRECHER *et al.,* 2000). In our country, none of the few commercial kits, either serological or based on histochemical techniques, are currently available in routine laboratories.

Conventional one-round PCR targeting different *Bartonella* genes such as the 60kDa heat-shock protein (ANDERSON *et al.,* 1994), the citrate synthase enzyme (NORMAN *et al.,* 1995), the 16S rRNA (BIRTLES, 1995), the 16S-23S intergenic spacer (MINNICK & BARBIAN, 1997) and the cell division protein FtsZ (KELLY *et al,* 1998) reported variable sensitivities, although some of the techniques were able to discriminate

(1) Clinical Laboratory, Children's Institute, *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo*, São Paulo, SP, Brazil. E-mail: karina.kawasato@icr.usp.br
(2) Medical Laboratory (LIM 03), *Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo*, São Paulo, SP, Brazil. E-mail: lealea@uol.com.br
(3) Laboratory of *Bartonella* Investigation, *Departamento de Clínica Médica da Faculdade de Ciências Médicas da Universidade Estadual de Campinas*, Campinas, SP, Brazil. E-mail: pvelho@unicamp.br
(4) Laboratory of Seroepidemiology and Immunobiology, *Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo,* São Paulo, SP, Brazil. E-mails: lidyam@ig.com.br and thelma.okay@usp.br
(5) Laboratory of Medical Mycology (LIM-53), Clinical Dermatology Division, *Hospital das Clínicas da FMUSP and Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo,* São Paulo, SP, Brazil. E-mail: gildamdn@usp.br
**Correspondence to:** Thelma Suely OKAY, MD, PhD, Associate Professor. Laboratory of Seroepidemiology and Immunobiology, *Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo,* Av. Dr. Enéas Carvalho de Aguiar 470, 05403-000 São Paulo, SP, Brazil. Phone: +55.11.3061 7022. Fax: + 55.11.3061 8270. E-mail: thelma.okay@usp.br

among species. More recently, a quantitative amplification targeting the *Bartonella* heat-shock protein groEL was used to investigate bacteremia levels in serum samples of 18 patients with CSD, however, positive results were detected in only three patients (VERMEULEN *et al.,* 2008).

In the present study, we standardized three nested-PCR targeting the 60kDa heat-shock protein (ANDERSON *et al.,* 1994), the *Fts*Z gene (KELLY *et al.,* 1998) and the 16S-23S intergenic spacer (MINNICK & BARBIAN, 1997) to improve *B. henselae* DNA detection in different biological specimen, including peripheral blood samples of immune competent and immune compromised patients.

## PATIENTS, MATERIAL, METHODS

This research was approved by the Institutional Ethics Committee (CAPPesq protocol number 1245/2006). Informed consent was obtained from all patients, their parents, or legal guardians.

Twenty three samples from 18 patients with clinical and laboratory (n = 5), or only clinical and/or epidemiological suspicion of bartonellosis (n = 13) were tested: 13 blood samples, seven lymph node biopsies, two skin biopsies and one lymph node aspirate.

Three milliliters of EDTA whole blood samples or 10 mg of skin, lymph node biopsies or aspirates were used for DNA extraction (QIAmp or DNeasy & Tissue kit, QIAGEN Inc., USA)., according to the manufacturer instructions.

Amplifications were performed following strict measures to minimize the risk of carry over contamination (BURKARDT, 2000). Briefly, reactions were set up in three separate rooms containing dedicated equipment, supplies and pipetting devices. Reagents and samples were handled with disposable gloves, DNA/RNase-free microtubes and pipetting tips in safety cabinets equipped with UV lights.

The outer primers (IDT, Iowa-USA) were designed elsewhere (ANDERSON *et al.,* 1994; MINNICK AND BARBIAN, 1997; KELLY *et al,* 1998) and the inner primers were designed in our laboratory. The nested-HSP was performed with the primers forward 5'- AGCTGGTATCAAGGCAGGTG-3' and reverse 5'- ATTTTCGTCTTCAGGCATCG-3' generating an amplification product of 184 bp; the nested-ITS was performed with the primers forward 5'- GAT GATGATCCCAAGCCTTC-3' and reverse 5'- TCCCC GGCATAATCTCATAA- 3'generating an amplification product of 113 bp, and the nested-FtsZ was performed with the primers forward 5'- CAAAACGGTTGGAGAGCAGT-3' and reverse 5'- CGCCTG TCATCTCATCAAGA-3' generating an amplification product of 218 bp. Amplifications were carried out in a T3 Thermocycler (Biometra, Göttingen, Germany), in a final volume of 50 µL containing 1,000 ng of genomic DNA in the first round and 10 µL were transferred onto the second round. Other reagent concentrations were: 200 µM of dNTP (Labtrade, São Paulo, Brazil), 2.5 U of Taq DNA polymerase (Labtrade, São Paulo, Brazil), 1.5 mM of $MgCl_2$ and 0.4 µM of each primer. Amplifications consisted of an initial denaturation step of five min at 95 ºC, 40 cycles of one min at 95 ºC, one min at 60 ºC for nested-HSP and nested-FtsZ or 55 ºC for the nested-ITS, one min at 72 ºC, ending with a final extension step of five min at 72 ºC. PCR products were detected in 2% agarose gels stained with 0.5 µg/mL of ethidium bromide and perceived by UV transillumination (Vilber Lourmat, France). In each experiment, *B. henselae* DNA extracted from a purchased ATCC 49882$^R$ strain (Adolfo Lutz Institute, São Paulo, Brazil) was used as the positive control, and two sterile deionized water samples, representing the master mix and the DNA extraction room were used as negative controls.

To determine the detection limit, serial dilutions of *B. henselae* DNA, ranging from 1 ng to 1 fg, were spiked with 1,000 ng of DNA from a healthy individual. Each dilution was tested 10 times to ensure reproducibility.

Microorganisms that can cause mononucleosis-like syndrome were included in the specificity evaluation because they can cause signs and symptoms that could be confused with the cat scratch disease or other acute infectious diseases: *Escherichia coli*, *Streptococcus pyogenes, Staphylococcus epidermidis, Chlamydia pneumoniae, Mycobacterium tuberculosis*, Cytomegalovirus, Epstein-Barr virus, *Toxoplasma gondii,* as well as four other *Bartonella* species: *B. quintana*, *B. elizabethae, B. vinsonii and B. clarridgeiae.* These microorganisms DNA were tested three times in different DNA concentrations (100 ng, 500 ng and 1,000 ng).

Considering that a laboratory investigation of *Bartonella* spp. infection was performed in only five of the 18 patients, the confirmation of amplification results was made using RFLP and DNA sequencing, planning to adopt the RFLP as the standard confirmatory method in the future due to its better cost-effectiveness. In the case of nested-FtsZ, 4 µL of the amplification products were digested with 5U of the restriction enzyme H*py*CH4IV (New England Biolabs, Ipswich, USA) in a final volume of 20 µL. After an incubation of three hours at 37 ºC, the *B. henselae* amplification product (218 bp) should generate two fragments of 127 and 91 bp.

The entire nested-FtsZ product was purified (QIAquick PCR Purification Kit, QIAgen) and sequenced twice in each direction (ABI-377, Applied Biosytems Incorporation, Foster City, California, USA). The degree of homology between amplified sequences and *B. henselae* Houston-1 strain (GenBank AF061746.1) was assessed by sequence alignment using the BLAST software available in http://www.ncbi.nlm.nih.gov/BLAST.

Sequence alignment was also used to ensure that the DNA sequence amplified by the nested-FtsZ did not present a significant degree of homology with the other *Bartonella* species: *B. elizabethae* (GenBank AF467760.1); *B. vinsonii subsp berkoffii* (GenBank AF467764.1); *B. clarridgeiae* (GenBank AF141018.1); *B. rochalimae* SM318006 (GenBank DQ676490.1) and *B. rochalimae* HUMBOLT 131 (GenBank DQ 676486.1).

Statistical analysis was performed by SPSS (version 13.0 for Windows, SPSS Inc; USA). The frequency of positive results was compared for each of the three systems after the first, and after the second round of amplification. Considering the three nested-PCR, the frequency of positive results was also compared. The agreement or disagreement of results was determined by the McNemar test and $p$ values $\leq 0.05$ were considered statistically significant. Bonferroni's correction was used for multiple comparisons ($p_c$).

## RESULTS

In the present study, three nested-FtsZ-PCR were developed to

improve the molecular detection of *Bartonella henselae* in different biological samples, with emphasis on peripheral blood.

Twenty three samples from 18 patients were tested. Demographic data, signs and symptoms, laboratory tests results, antibiotic therapy and outcome of patients were summarized in Table 1. As an overview, 11 patients were female, ages varied from two to 80 years old, 15 had received antibiotics, 10 had a fever, 10 confirmed they had contact with cats, nine had adenomegaly, eight had underlying diseases, and in two patients the disease led to death. The cases of patients numbered 1 and 3 were reported elsewhere (VELHO *et al.,* 2007; MAGALHÃES *et al.,* 2010).

The limiting dilution test revealed that the first round of amplifications for each of the three systems (HSP, ITS and FtsZ) consistently detected 1 pg of *B. henselae* DNA, and the second round 100 fg. There was no cross reactivity with the microorganisms used in the specificity tests, including DNA of other three species of *Bartonella*: *elizabethae; vinsonii subsp berkoffii; clarridgeiae.* However, the nested-HSP and the nested-ITS yielded a positive, although weak, amplification when the higher concentration (1,000 ng) of *Bartonella quintana* was tested, even though the primers design was aimed at amplifying only the species *henselae*.

The first amplification was positive in one sample by HSP (1/23 or 4.3%); in four samples by FtsZ (17.4%), and in five samples by ITS (21.7%). After the second round, there were six positive samples by nested-HSP (26%), and eight by nested-ITS (34.8%), while the nested-FtsZ was able to detect 18 samples (78.2%): 10 peripheral blood samples, five lymph node biopsies, two skin biopsies and one lymph node aspirate. Statistical analysis comparing the frequency of positive results after the first and the second round of amplification confirmed that the nested amplification was worth performing only for the nested-FtsZ (4/23 or 17.4% *vs.* 18/23 or 78.3%; $p = 0.0001$), whereas there was no statistical significance for the nested-HSP ($p = 0.063$) or the nested-ITS ($p = 0.250$). Moreover, among the three amplification systems, the nested-FtsZ proved to be more sensitive than nested-HSP and ITS ($p < 0.0001$).

RFLP results after digestion of the FtsZ amplification products with the enzyme H*py*CH4IV are displayed in Figure 1. As expected, all 18 positive samples generated two fragments of 127 and 91 bp.

In addition, automated DNA sequencing has confirmed RFLP results, i.e., the 18 amplification products were from the *B. henselae* FtsZ gene, as

**TABLE 1**
Demographic and epidemiological data, signs/symptoms, laboratory investigation, treatment and outcome of the 18 patients included in the study

| N° | A | G | Underlying disease | Signs, symptoms and epidemiological data | Samples tested by PCR | Laboratory investigation | Antibiotics | Outcome |
|---|---|---|---|---|---|---|---|---|
| **1*** | 36 | M | Intravenous drug addict, AIDS | Fever. pulmonary edema, bacillary angiomatosis, severe anemia, hepatitis, peritonitis, pleuritis, pericarditis, perianal lesion, and skin papule. Contact with cats and dogs. | skin biopsy (papule) | Electron microscopy of the papule biopsy showed a double-layered-bacterium adhered to an erythrocyte. Histopathology was suggestive of *Bartonella* spp. Negative blood and skin cultures. Negative anti-*B. henselae* and *B. quintana* serology (IFAT-IgG). Positive PCR of the papule. DNA sequencing confirmed that PCR amplified *B. henselae* DNA. | clarithromycin | Recovery of acute symptoms. The patient moved to another state and he was lost to follow-up. |
| **2** | 14 | F | none | No fever. Angio-proliferative skin lesion. Contact with cats. | blood | Electron microscopy of the skin biopsy showed a double-layered-bacteria adhered to erytrocytes, Positive anti-*Bartonella* IgG *(IFAT).* The blood sample yielded a positive nested-FtsZ-PCR. The skin lesion was not tested by nested-FtsZ-PCR | Erythromycin | Full recovery |
| ***3** | 27 | F | none | Asymptomatic. Had donated blood (Public Blood Bank). Contact with cats. This woman is the mother of patient number 4. | Blood | Optical microscopy of the erythrocytes from the blood bag showing bacteria adhered to erythrocytes. Blood was positive by nested-FtsZ-PCR. | None | Outpatient clinic follow-up with negative nested-FtsZ-PCR in peripheral blood after six months |
| **4** | 3 | F | Neonatal cholestasis syndrome | No fever. anemia, jaundice, hepatosplenomegaly. Contact with cats. | Blood | IFAT -IgG was negative for *Bartonella henselae* and *B. quintana*. Electron microscopy showed double layered- bacteria adhered to erythrocytes. The child peripheral blood at three years old was positive by nested-FtsZ-PCR. | Erythromycin, gentamycin and azythromycin | Improvement of infection and outpatient clinic follow-up |

**TABLE 1**
Demographic and epidemiological data, signs/symptoms, laboratory investigation, treatment and outcome of the 18 patients included in the study (continuation)

| N° | A | G | Underlying disease | Signs, symptoms and epidemiological data | Samples tested by PCR | Laboratory investigation | Antibiotics | Outcome |
|---|---|---|---|---|---|---|---|---|
| **5** | 11 | M | None | Fever, hepatomegaly | Blood | Blood culture identified *Bartonella* spp. Peripheral blood was positive by nested-FtsZ-PCR. | Amikacin and cefotaxime | Full recovery |
| **6** | 5 | M | None | Fever, cervical adenomegaly. | Lymph node biopsy, blood | Lymph node biopsy and peripheral blood were positive by nested-FtsZ-PCR. | Cotrimoxazole | Full recovery |
| **7** | 5 | M | None | Fever, axillary adenomegaly. Contact with cats. | Blood | Peripheral blood was positive by nested-FtsZ-PCR. | Cotrimoxazole | Full recovery |
| **8** | 9 | F | None | High fever for 3 months and cervical adenomegaly. | Lymph node biopsy | Lymph node biopsy was positive by nested-FtsZ-PCR | Claritromicin | Full recovery |
| **9** | 8 | F | None | No fever, cervical adenomegaly. Contact with cats. | Lymph node aspirate | Lymph node aspirate was positive by nested-FtsZ-PCR. | None | Full recovery |
| **10** | 9 | F | None | Fever, contact with cats and cat scratch | Lymph node biopsy, blood | Lymph node biopsy and peripheral blood were positive by nested-FtsZ-PCR. | Cotrimoxazole Cefalexin | Full recovery |
| **11** | 6 | F | None | Fever, Contact with cats. and antecedent of cat scratch two months before | Blood | Peripheral blood was positive by nested-FtsZ-PCR. | Cotrimoxazole | Full recovery |
| **12** | 12 | M | None | No fever, cervical adenomegaly. | Lymph node biopsy | Lymph node biopsy was positive by nested-FtsZ-PCR | Cotrimoxazole | Full recovery |
| **13** | 9 | F | Meningo encephalitis and seizures. | Two months of fever and axillary adenomegaly. Contact with cats. | Blood | Peripheral blood was positive by nested-FtsZ-PCR. | Amoxycillin, cefalexin, erythromycin | Full recovery |
| **14** | 7 | M | AIDS, peliosis hepatis | High and persistent fever, negative blood, urine and CSF cultures. | Skin biopsy | Skin biopsy was positive by nested-FtsZ-PCR. | Vancomycin, clindamycin and doxycycline | Death |
| **15** | 12 | M | Chronic Granulomatous Disease | No fever, cervical adenomegaly. | Lymph node biopsy, and blood | Lymph node biopsy and peripheral blood were positive by nested-FtsZ-PCR. | Cefalexin | Death |
| **16** | 61 | F | Hodgkin lymphoma | No fever. Cervical and inguinal adenomegaly. Contact with cats | Lymph node biopsy, blood | Lymph node biopsy and peripheral blood were negative by nested-FtsZ-PCR. | None | Improvement of symptoms, under chemotherapy |
| **17** | 80 | F | Non-Hodgkin lymphoma | No fever. Cervical adenomegaly, pneumonia and pleural effusion | Lymph node biopsy and blood | Lymph node biopsy and peripheral blood were negative by nested-FtsZ-PCR. | Ciprofloxacin | Improvement of symptoms, under chemotherapy |
| **18** | 2 | F | Liver insufficiency, portopulmonary hypertension | Fever and splenomegaly | Blood | Staphylococcus coagulase negative was isolated from blood. Peripheral blood sample was negative by nested-FtsZ-PCR. | Cefepim | Improvement of symptoms. Outpatient clinic follow-up. |

A = age in years; G = gender (masculine or feminine). * reference VELHO *et al.*, 2007 (case No. 1) and MAGALHÃES *et al.*, 2010 (case No. 3).

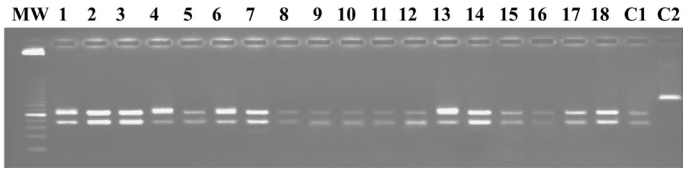


**Fig. 1 -** RFLP of the 18 positive nested-FtsZ-PCR samples (10 blood samples, 5 lymph node biopsies, 2 lymph node aspirates and 1 skin biopsy) after digestion with *Hpy*CH4IV (lanes 1 to 18). The molecular weight marker (MW) is the 25 base-pair ladder (Invitrogen) and the strongest band is 125 base-pairs. C1 displays the amplification product of the Houston-1 strain after digestion (positive control), generating two fragments of 127 and 91 base-pairs, like the 18 positive samples of the study, and C2 exhibits the same positive control prior to *Hpy*CH4IV digestion showing the entire fragment of 218 base-pairs.

the degree of homology between these 18 PCR products and *B. henselae* Houston-1 strain varied from 97 to 100% (data not shown).

## DISCUSSION

*Bartonella* spp. isolation and specific IgG detection is available in just a few university centers in our country, and therefore in most cases the diagnosis of bartonellosis is based on the association of suggestive signs and symptoms with at least one of the following parameters: epidemiological data reporting contact with animals, especially cats; microscopy and/or histological findings (HANSMANN *et al.,* 2005).

Due to the fact that our patients live in areas with low levels of laboratory resources, in a significant amount of cases none of the specific tests to diagnose *Bartonella* spp. infections were performed. In order to circumvent the lack of a gold standard in these cases, we confirmed that the nested-FtsZ amplifications corresponded to *B. henselae* DNA by using RFLP and DNA sequencing. Due to budget constraints, we did not sequence the six nested-HSP positive samples and the eight nested-ITS ones.

It is known that the target gene, the type of biological sample, the sample size as well as different DNA extraction protocols can dramatically influence amplification results. Only a few studies have reported detection of *Bartonella* spp. DNA in blood samples of CSD or immune compromised patients (ARVAND *&* SCHÄND, 2006; VELHO *et al.,* 2007), while the detection in serum samples has so far been unsuccessful (VERMEULEN *et al.,* 2008).

Although the HSP gene primers had already been used to amplify lymph node biopsies and secretions by ANDERSON *et al.* (1994); AVIDOR *et al.* (1997) and HANSMANN *et al*. (2005) with detection rates varying from 69 to 84%, our nested-HSP was the least sensitive of the three systems, and yielded positive results with *B. quintana* DNA, even though the primers were designed to amplify only *B. henselae*.

GARCIA-ESTEBAN *et al.* (2008) reported the detection of *Bartonella* by a 16S-23S rRNA intergenic spacer and a multiplex-PCR targeting the 16S rRNA gene combined with a reverse line blotting that could simultaneously detect around 20 different *Bartonella* species from a number of clinical specimens. In the present study, despite the use of a new set of primers of the 16S-23S rRNA intergenic spacer in nested amplifications, the sensitivity was low, and there was cross-reactivity with *B. quintana* DNA, as in the case of the nested-HSP amplifications.

EHRENBORG *et al.* (2000) used different primers of *B. henselae* FtsZ gene in one-round amplifications. They tested 15 biological specimens including tissue fragments and blood samples from cats and humans, either HIV-positive or with no underlying conditions, and found a positivity of 64%.

The C-terminal end of the FtsZ gene was used to investigate the presence of *Bartonella* spp. DNA in 80 samples (lymph node biopsies, lymph node aspirates, heart valve fragments) of patients with CSD and/or endocarditis by a one-round PCR. They detected 35 positive samples (43.75%) in comparison with 39 positive samples (48.75%) by ITS or pap31 one-round amplifications (ZEAITER *et al.,* 2002). In the present study, the same FtsZ gene region was used to test 23 samples of 18 patients, 15 of whom had already received antibiotics, but even so the nested-FtsZ-PCR was positive in 18 samples of 15 patients. ARVAND & SCHÄD (2006) reported detection of *B. henselae* DNA in a child with CSD up to nine weeks after admission and three weeks of antibiotic therapy. In this child several blood samples were tested, and they used a semi-nested PCR targeting the *B. henselae* htrA gene.

Among the five patients with laboratory evidence of *Bartonella* spp. infection (1-5, Table 1), PCR confirmed the diagnosis in all of them (four blood samples and one skin biopsy). Patient number one had AIDS and patient number 4 was a three year old child with an antecedent of neonatal cholestasis syndrome, while the other three patients had no underlying conditions.

The two skin biopsies analyzed in the present study were positive by PCR and belonged to AIDS patients (Table 1, number 1 and 14). With regards to the seven lymph node biopsies, PCR was positive in five, and only one of seven patients (number 15) had a previous medical condition. In two patients only the lymph node biopsy was analyzed (number 8 and 12), and in three patients blood samples were tested in parallel and all specimens were positive (number 6, 10 and 15).

As for the 13 blood samples analyzed in the study, 10 were positive (seven of 10 patients had no underlying conditions). The three negative blood samples belonged to patients 16 and 17, who were later diagnosed with lymphoma, and to patient number 18 who had a staphylococcal infection detected by blood culture. Nevertheless, considering that our nested-FtsZ is species-specific, it is not possible to rule out the presence of other *Bartonella* species in these patients.

We conclude that nested amplifications increased the detection of *B. henselae* DNA, and that nested-FtsZ was the most sensitive and the only one specific to *B. henselae* in different biological specimens. Taking into account that all samples detected by nested-HSP and nested-ITS were also detected by the nested-FtsZ, and that the 18 positive samples presented with highly homologous nucleotide sequences in comparison with *Bartonella henselae* Houston-1 strain, we infer that in our series all infections were caused by *Bartonella henselae*. The high positivity of blood samples has highlighted the use of this biological material to investigate bartonellosis irrespective of the patients' immune status. This is of great importance in critically ill patients and in young children where this method can be used in order to avoid more invasive procedures such as lymph node biopsies and fine-needle aspirates. Considering that nested-amplifications are more prone to carry-over contamination, we intend to develop a quantitative diagnostic tool based on the primers presented herewith.

## RESUMO

### Detecção de DNA de *Bartonella henselae* em amostras clínicas, incluindo sangue periférico, de pacientes imunocompetentes e imunodeprimidos por meio de três amplificações duplas

Bactérias do gênero *Bartonella* constituem patógenos emergentes detectados em biópsias de linfonodos e secreções de gânglios provavelmente devido a maior concentração de bactérias. Vinte e três amostras de 18 pacientes com dados clínicos, laboratoriais e/ou epidemiológicos sugestivos de bartonelose foram submetidas a três amplificações duplas para a detecção de fragmento da proteína de choque térmico de 60-kDa (HSP), do espaçador interno 16S-23S rRNA (ITS) e da proteína de divisão celular (FtsZ) de *Bartonella henselae,* para melhorar a detecção em amostras clínicas. Na primeira amplificação, uma, quatro e cinco amostras, respectivamente, foram positivas pelo HSP (4,3%), FtsZ (17,4%) e pelo ITS (21,7%). Com a segunda amplificação foram identificadas seis amostras positivas pelo nested-HSP (26%), oito pelo nested-ITS (34,8%) e 18 pelo nested- FtsZ (78,2%), correspondentes a 10 amostras de sangue periférico, cinco biópsias de linfonodos, duas biópsias de pele e um aspirado de gânglio. A nested-FtsZ foi mais sensível que a nested-HSP e a nested-ITS ($p < 0,0001$), possibilitando a detecção de DNA de *Bartonella henselae* em 15 de 18 pacientes (83,3%). No presente estudo, três nested-PCR, consideradas específicas para a amplificação da *Bartonella henselae,* foram desenvolvidas, porém somente a nested-FtsZ não amplificou o DNA de *Bartonella quintana*. Concluímos que amplificações duplas aumentaram a detecção de DNA de *B. henselae,* e que a nested-FtsZ foi a mais sensível e a única específica para *B. henselae* em diferentes amostras biológicas. Como todas as amostras detectadas pelo HSP-nested e nested-ITS foram também pela nested-FtsZ, inferimos que, em nossa casuística, as infecções foram causadas por *Bartonella henselae*. A elevada positividade de amostras de sangue chamou a atenção para a utilização deste material biológico na investigação de bartoneloses, independentemente do estado imune dos pacientes. Este fato é importante no caso de pacientes criticamente enfermos e crianças pequenas para evitar procedimentos mais invasivos, como biópsias e punções de gânglios.

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